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CONTENTS ON INSIDE COVER

Including ALLERGY ABSTRACTS

The Journal of Allergy

CONTENTS FOR MARCH, 1945

American Academy of Allergy
First Annual Meeting, New York, N. Y.
Dec. 11 and 12, 1944

The Relationship of Allergy to Medicine. David P. Barr, M.D., New York, N. Y. ---- 61

The Significance of Histamine in Anaphylaxis. Carl A. Dragstedt, M.D., Chicago, Ill. ---- 69

Nutritional Aspects of Globulin Metabolism. Paul R. Cannon, M.D., Chicago, Ill. ---- 78

Treatment of Urticaria With Synthetic Vitamin K. J. H. Black, M.D., Dallas, Texas ---- 83

Moisture Characteristics of Pollen. Arthur B. Berresford, M.D., and Robert A. Cooke, M.D., New York, N. Y. ---- 87

Studies in Food Allergy. IV. Variations in Allergenic Activity of Food Extracts. Louis Tuft, M.D., George I. Blumstein, M.D., and L. J. Wenger, M.D., Philadelphia, Pa. ---- 92

Original Articles

Air-Borne Fungi in Allergic Disease. I. A New Method of Preparation of Mold Extracts; Incidence of Skin Reactions With Mold Extracts. Major Stanley F. Hampton, M.C., Army of the United States, and Lieutenant Edwin P. Lowe, A.C., Army of the United States ---- 101

Editorial

Allergy at the Crossroads ---- 109

On the Standardization of Extracts ---- 111

American Academy of Allergy

Presidential Address. Robert Chobot, M.D., New York, N. Y. ---- 112

Transactions of the Annual Meeting ---- 116

Notes ---- 120

Allergy Abstracts

Asthma and Hay Fever—Dermatology—Otorhinology and Ophthalmology—Miscellaneous Allergies—Anaphylaxis—Miscellaneous --17-32

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The Journal of Allergy

VOL. 16 MARCH, 1945 NO. 2

American Academy of Allergy

First Annual Meeting, New York, N. Y., Dec. 11 and 12, 1944

THE RELATIONSHIP OF ALLERGY TO MEDICINE

DAVID P. BARR, M.D., NEW YORK, N. Y.

UNTIL 1900, medicine was studied and practiced without knowledge of allergy, of hypersensitiveness, or of anaphylaxis. Indeed the concepts, the name, and the application of allergy have been products of the twentieth century and, for the most part, of the last forty years. Those of us who have gathered here today may wonder what medicine or its practice could have been without the light of allergy. Actually, many significant though isolated facts were well known concerning the principal diseases now customarily included in the province of the allergist.

Medicine Without Allergy.—Asthma had successively attracted the attention of such master clinicians as von Helmont, Willis, Floyer, Cullen, and Laennec. Many of its essential aspects had been recognized: its paroxysmal character with the variable intervals of freedom from any anatomic or physical signs of disease, its preponderant incidence before the age of 40 years, its hereditary tendency and its greater frequency among the more well-to-do. In the midst of theories of its supposed nervous origin, Henry Hyde Salter, in 1859, had called attention to the fact that asthmatic paroxysms could be provoked by particular kinds of air, by chemical and mechanical irritants, and by animal emanations. He gave in some detail an account of a certain asthmatic who was obliged to take a sea voyage during the hay fever season. He also told the story of a patient who could not stroke or nurse a cat without exciting a paroxysm of dyspnea. Before the end of the nineteenth century, many exciting causes of asthma had been recognized, and textbooks contained the information that animal emanations, certain plants, and many pollens might furnish the immediate cause for an attack.

More exact data had been accumulated concerning hay fever. As early as 1819, Bostock had described the condition under the name of summer catarrh and had advanced the hypothesis that the symptoms

Read at the First Annual Meeting of the American Academy of Allergy, New York, Dec. 11, 1944.

were concerned with vegetation and in particular with hay. In 1872, Norrel Wyman, of Boston, had described typical hay fever due to ragweed, and in the following year Blackley had demonstrated that a drop of pollen extract on the scratched arm of a sensitive subject produced a local irritative reaction.

One of the most significant contributions to the subject later to be known as allergy had been made by Sir William Osler. Beginning with the year 1894, he reported cases of diseases of the skin characterized by exudation of blood or serum and by a variety of visceral manifestations. These included simple erythema, exudative erythema, erythema nodosum, urticaria, certain purpuras, and angioneurotic edema. Osler emphasized the similarity of the conditions under which the various eruptions occur; the frequency with which the lesions were substituted, one for the other in the same patient at different times; the tendency to recurrence often over many years; and the identity of the visceral manifestations which accompanied the different eruptions. He enumerated a wide variety of factors which appeared as exciting causes, and included cholelithiasis, cirrhosis, malarial chills, ingestion of shellfish and of strawberries, and hydatids. He also mentioned the urticaria occasionally seen after exposure to cold. He was impressed with certain similarities between the erythema group of diseases, and paroxysmal asthma.

In spite of its range and extensiveness, clinical knowledge of asthma, hay fever, and the erythema group of skin diseases in 1900 had led to no clear idea of their nature. The significance of the multitudinous causes of asthma lay hidden under the term idiosyncrasy, and was not sufficiently appreciated to shake the belief of physicians that the disease was a diathetic neurosis. The fine observations of Bostock, Wyman, and Blackley had meant little because at the time they led to no cure and failed to explain why many exposed to the flowers of grasses and the pollen of ragweed did not suffer from hay fever. Even Osler's exact and illuminating clinical notes led neither to clear understanding of the relationship of the various diseases nor to a constructive etiological approach in diagnosis or treatment.

In considering relevant knowledge at the end of the nineteenth century one must not fail to mention the status of the embryonic science of immunology, which was then regarded as an entirely separate and unrelated field. Sewell's classical observations on snake venom had led successively to Calmette's production of antivenom, to Roux's studies of diphtheria toxin and to von Behring's brilliantly successful diphtheria antitoxin. The clinical use of horse serum antitoxin had disclosed a curious though troublesome complication. It had been found that guinea pigs receiving toxin-antitoxin mixtures in the standardization of diphtheria antitoxin could not be used for subsequent testing because many of them died quite suddenly and unaccountably following repeated injections of horse serum. Furthermore, it was known that

mysterious and alarming symptoms occasionally developed in patients who were receiving diphtheria antitoxin.

The Contributions of Allergy.—This was the state of affairs when, at the turn of the century, Richet undertook the study of reactions which ensued after injection of antitoxin. Richet's discovery and his enunciation, in 1902, of the basic principles of anaphylaxis marked the birth of allergy. It was only three years after Richet's report that Weichardt and, almost simultaneously, Wolff-Eisner proposed that hay fever might be a manifestation of anaphylaxis. The suggestion that asthma might be similarly classified awaited the demonstration by Auer and Lewis that anaphylactic reactions were accompanied by bronchospasm. The practical significance of the concept became more apparent as other phenomena of anaphylaxis were disclosed. It appeared that the offending substances or antigens determining the anaphylactic response were proteins or compounds of other substances with proteins; that the reactions to such antigens depended upon the production of antibodies; that the reactions varied in different species and in individuals of the same species; that, regardless of species, the most striking components of the anaphylactic reaction in all animals were smooth muscle spasm and an increased capillary permeability; and, finally, that reinjections of sublethal amounts of the antigen might result in temporary, more or less complete desensitization or immunity. These characteristics had so many counterparts in the clinical behavior of hay fever and asthma that there could be little doubt as to their close relationship. Very early however there were observed certain apparently significant differences between the anaphylaxis of animals and the human reactions to foreign proteins; among them the heritable character of hay fever and asthma; the difficulty of producing intentional sensitization; and the frequent failure to demonstrate antibodies or previous exposure in cases of human sensitization. These differences have not altered the practical importance of the concept but have emphasized the utility of von Pirquet's definition of allergy as an altered capacity of tissues to react to bacterial and nonbacterial substances after their invasion of the body and the later broadeninng of the term allergy to include bacterial hypersensitiveness in man and in animals as well as all forms of nonbacterial hypersensitiveness.

The idea of allergy once grasped by physicians was found to be applicable to one clinical condition after another. Erythemas, urticaria, angioneurotic edema, and several forms of purpura were successively added to the list of conditions dependent at least in part upon sensitiveness to proteins. Allergy was shown to play a role in certain forms of headaches and even to be the cause of some convulsive seizures. The work of Landsteiner indicated that many examples of idiosyncrasies to drugs could be explained on an understandable allergic basis while the observations of Rackemann, Harkavy, and Rich showed its possible relationship to periarteritis nodosa and other diseases of the blood vessels.

It was also found that while many individuals displayed major clinical manifestations, there were immensely more people who suffered temporarily or in a minor way from allergic symptoms.

If one takes into account only the major forms of allergy such as asthma, hay fever, eczema, urticaria, and headaches, a conservative estimate indicates that many more than ten million of the people of the United States suffer from the condition. If minor forms are included, or if the newer concepts of drug allergy and arterial hypersensitiveness are accepted, the number involved is immensely greater. Moreover, it must be apparent to all that the concept of allergy is much more inclusive than is indicated by the above lists. It is illuminating that von Pirquet's original experiments were made with tuberculin, and that his first definition included the idea of altered reactivity of the body to bacteria. The essential similarity of immunity and of allergy, at first regarded as antitheses, has become more apparent with increasing knowledge. It is now understood that for the production both of immunity and of allergy an antigen is essential; that this is a foreign protein which may be either a product of a living organism, toxic or otherwise, or a protein or protein-linked substance unassociated with a living organism. The introduction of such protein is followed by the formation of specific antibodies which in so far as they involve cells, give to them an altered reaction to the specific antigen. Under such circumstances allergy is present, and allergic reactions result whenever the specific antigen is introduced in appropriate amounts. As Cohen points out, this general formula may be applied to almost any infectious process, with the understanding that in the infectious disease the antigens may be either specific toxins elaborated by bacteria or substances which arise in the body in response to the introduction of any foreign protein. The degree of reaction would appear to depend on the amount of the toxic material or antigen and also upon the quantitative relationships between antigen and antibody. From this point of view many immunologic phenomena may be regarded as allergic in the sense that they represent an altered reaction of cells to a specific antigen, and that the quantitative relationships of antigens and antibodies determine the recognizable existence and degree of sensitivity or immunity.

Allergy as a Specialty.—A result of this great development is the specialty of allergy, a branch of medicine to which men of ability are now willing to devote their lives. Everyone may deplore departmentalization and specialization in medicine. All realize that each division of the broad field tends to restrict the outlook both of those who do, and of those who do not, take part in a specialty. Because, however, of our limited capacity, some specialization must be regarded as essential. It may be regarded as justifiable whenever the knowledge involved in the practical application of a subject is so vast that only those who devote to it their entire time may master it or whenever the technical procedures involved in its practical application are so intricate or difficult that only

those constantly using them can perform them adequately. From both points of view there appears to be justification for specialists in allergy. The immunologic background of anaphylaxis and hypersensitiveness has been so complex and has developed with such rapidity that no man can say that he has mastered it. The practice of allergy on the other hand has been wrapped up with the application and interpretation of the skin test, with ophthalmic reactions, with patch tests, with passive transfer, and with the evaluation of such laborious and disappointing tests as the leucopenic index. It has been involved with the preparation, purification, and fractionation of antigens. Above all, it has been concerned with the practical detective work involved in discovering from detailed history of background and environment the essential factors responsible for a patient's hypersensitiveness. The many practical and theoretical contributions which have been made by allergists as well as the relief which has been experienced by innumerable patients as the result of their efforts have more than justified the specialization.

It must be admitted, however, that in the development of the still very young specialty of allergy some quite undesirable situations have arisen.

1. Some allergic practice has tended to stray away from the fundamental principles of general medicine. Since mastery of so complicated a field tends to absorb the entire time and attention of those engaged in the study, it is perhaps not surprising that the attention of some allergists has been undesirably withdrawn from other aspects of diagnosis and treatment. It is less understandable when this tendency has led to neglect of history and examinations which do not bear directly on hypersensitiveness.

2. The field itself has become, in actual practice, too restricted. The emphasis on the clinical study of hay fever and asthma has tended to limit the scope of those specially trained in the principles of allergy and to such an extent that one finds dermatologists taking over the allergy of the erythema group, pediatricians attacking the problem of infantile eczema, and immunologists and bacteriologists in medicine appropriating the allergy of infectious disease. If allergy is a specialty, it should be such on the basis of the principles which underlie the subject and not on the basis of specific testing and treatment of any one or any isolated group of the diseases to which it pertains.

3. There have been among some allergists in certain quarters a too uncritical appraisal of the methods of diagnosis and treatment. There has been a failure to appreciate the many pitfalls of the skin test and a tendency to ignore the variations which may arise from insufficient standardization in the preparation of allergens as well as in the technique and in the reading of the tests. Furthermore, in the specialty of allergy ideas and theories have been accepted too readily as facts, and extravagant and unjustified claims have been made both for the scope of allergy and the benefits to be derived from its practice. Insofar as

these situations have obtained, they have tended to discredit the specialty with other members of the profession.

4. The development of the specialty has tended to deprive other practitioners of adequate participation. The intricacies of allergic examination as well as the experience and the high degree of detective ability required for allergic diagnosis have discouraged many in general medicine from practicing even the elements of one of the most important branches of their art. It is more than deplorable that many young internists have been permitted to finish their training almost without contact with allergy and allergic thought. Some of them have been so crassly ignorant as to regard the whole specialty as a somewhat unnecessary and undesirable fad.

Certainly the concept of altered reaction to bacterial and nonbacterial substances is one of the most important ideas of medicine today. It ranks with psychosomatic medicine, with the study of the circulatory apparatus, with bacteriology, with metabolism, and with clinical chemistry. It is relevant to the pathogenesis and affects the practical management of much more than half of all the diseases to which flesh is heir. It is part and parcel of internal medicine and no internist can be regarded as completely trained who is not thoroughly cognizant of the principles of sensitivity, of anaphylaxis, and of the altered reaction of the body to proteins. On the other hand, no allergist can do justice to his patients or to his specialty who is not fundamentally trained in internal medicine to the extent that he uses habitually a comprehensive history, performs routinely a complete and searching examination, and is aware of related circulatory, respiratory, metabolic, and psychosomatic problems. Everywhere the practice of allergy impinges on other fields of medicine. In consideration of asthma one must take into account emphysema, bronchitis, the state of the circulation, and the state of the kidneys, as well as the constitution and psychological state of the patient. In dealing with hay fever, the allergist must be well acquainted with the problems of nasal infection, of sinusitis, and of other diseases of the upper respiratory tract. In considering the erythema group he must be aware of a great number of diseases of the skin with which the members of this group may be confused. In the more recent extensions of drug allergy, he must be aware of many of the problems of the chemistry, pharmacology, and clinical use of drugs. Tobacco allergy and the recently emphasized blood vessel allergy involve the field of peripheral vascular disease as well as acute rheumatic fever, periarteritis nodosa, and that strange group of conditions now sometimes collected under the name of angiitis.

Among the leaders in allergy there has never been any deviation from the principle that allergy and internal medicine are one and inseparable. It is interesting to note the list of those twelve men who met on March 7, 1924, in Robert Cooke's office to form the Association for the Study of Allergy. Among them were such notable internists as

Warfield Longcope, George MacKenzie, Francis Rackemann, Oscar Schloss, and Harry Alexander. The societies representing allergy have maintained for the past fifteen years one of the most effective special journals, the editorial policy of which has always been marked by strict adherence to scientific values and to the highest ideals for the specialty. A great step in advance was taken in 1943 when it was decided that those aspiring in future to the name of allergist should be first certified as internists, and as such should have experienced the fundamental training necessary for the understanding and practice of medicine as a whole. This emphasized as perhaps nothing else could the essential unity of allergy and internal medicine. Certainly nothing could have been more soundly helpful in developing and protecting the progress of the specialty of allergy. It was further specified that those who wished to be certified as allergists should have spent two full years in a satisfactory allergy clinic and its hospital and should have had training both in allergy and in internal medicine. Alternatives were offered, such as one year of full-time work of such character with two years additional in full attendance on an allergy clinic and its activities or five years' full attendance on an allergy clinic or special training under qualified preceptors solely, or in combination with, the other types of training.

It is one thing to make such requirements and another to render them just and effective. Some of those who have contributed most to the clinical practice and indeed to the fundamental understanding of allergy have certainly never enjoyed the training which is contemplated for the allergist of the future. Yet many of these same men would be named by any competent group of allergists as among the most desirable teachers of the young men now about to be trained. The other outstanding difficulty is the dearth of places for competent education of those who wish to become allergists. In a list published in THE JOURNAL OF ALLERGY in 1940, it appeared that there were available some thirteen residencies and fellowships. In addition there was a fairly long list (fifty-four) of clinics where men might work on two or more days a week. As far as one could see, the sole criterion of the eligibility of such clinics was their association with an approved hospital. No curriculum of instruction, no regimen of training, no specification as to how a man might spend his time was available.

The Training of an Allergist.—It is obvious that the training of a man intending to go into allergy should be intensive and yet along the broadest lines. It should include very thorough acquaintance with the principles and methods of immunology not only as they apply to so-called allergic diseases but also in their relation to infections. It should include a critical knowledge based on practice and experience of the immunologic tests which are clinically applicable. Ideally this should apply to tests used both in bacterial and nonbacterial allergy. It should include acquaintance with the preparation of allergens and knowledge of its difficulties. A considerable amount of time should be spent in the

dermatological clinic where the recognition of allergic conditions and their differentiation from nonallergic states could be emphasized. Acquaintance with nasal pathology and its relation to the allergic nose is more than desirable. The allergy clinic where the training is given should be conducted in the closest associations with a department of general medicine and the routine procedures of the clinic should not differ greatly from those of other diagnostic and treatment clinics in internal medicine. Last, but by no means least, the training should be conducted under much more than casual supervision by a master of the art aided by a group of men whose experience exceeds in general or in detail that of the candidate. The training should be stimulating to the degree that it would lead the candidate to investigation of unsolved problems in the field.

It is more than doubtful whether such a program can be completed adequately in less than two years of intensive training. One must emphasize, however, that the time spent is not the important factor. Of what value is an exposure for five years to a loosely run reference clinic where the principles of medicine are neglected, where the skin tests and other tests are employed uncritically, where observation of reactions is left largely to nurses and to nontechnical assistants, and where the time allowance for each patient is insufficient for that painstaking searching detective work which is the essence of allergic diagnosis? Such expenditures of time may result only in the acquisition of careless habits and of a false sense of competence. As in any other training in medicine the sound education of an allergist will depend upon the accumulation of large experience under expert guidance. It is high time that departments of medicine recognize the need of such facilities and organize them in such a way as to permit systematic and enlightened training. The pressure which is now being exerted by the Academy of Allergy and by other organizations interested in allergy must be met by equal efforts on the part of educational institutions.

It is only through training of the highest type that the extraordinarily important principles of allergy can be properly exploited, or that the practice of allergy can be made worthy of the subject. The allergist of the future should be the internist who, in addition to his sound clinical accomplishments, has acquired deep and fundamental knowledge of immunology. He should be the consultant whose advice will be sought in connection with immunologic problems whether they concern infectious or noninfectious disease. He should be the internist who possesses those special skills necessary for the recognition of sensitization and the detection of its cause. Last, it will be to him that we must look for research and for significant contributions to the great field of immunology in medicine.

THE SIGNIFICANCE OF HISTAMINE IN ANAPHYLAXIS*†

CARL A. DRAGSTEDT, M.D., CHICAGO, ILL.

THE significance of histamine in anaphylaxis has been discussed many times. This would not be the case if the question were settled beyond dispute or if the question had no inherent importance. A vast amount of effort is currently being expended in the practical matter of the control or cure of the allergic diseases in man, which is based upon the fundamental concept that histamine is causally related to the phenomena of anaphylaxis, and the assumption, by analogy, that it is likewise causally related to the phenomena of allergy. It is a matter of much practical as well as academic importance, therefore, that we review the evidence that has been presented which involves histamine.

There is a legitimate basis for the skepticism regarding the role of histamine in anaphylaxis in view of the following considerations. First, histamine has been accused at one time or another of being the toxin of intestinal autointoxication, the toxin of surgical shock, the cause of peptic ulcer, the toxemic factor in burns, and the toxin in the toxemias of pregnancy; to mention only some of the major indictments. It strains one's credulity to believe that a single agent with a highly characteristic pattern of performance could be responsible for such diverse disturbances, and in a measure the validity of each separate accusation seems to be diminished as the number and diversity of the accusations increase. Second, and perhaps more important, histamine has not been caught red-handed at the scene of the crime in that it has not been isolated in crystalline purity (finger-printed as it were) in association with anaphylactic phenomena. Investigators have talked about H-substance, histamine-like-activity, histamine-equivalent, etc. Some critics construe these scientific discretions as evasions and consider the validity of the indictment against histamine weakened thereby. There are other less cogent reasons for doubting the relationship of histamine to anaphylaxis that have been raised by various skeptics, some of which will be dealt with later.

The bill of indictment against histamine in anaphylaxis consists of three chapters. The first chapter deals with the plausibility and congruity of the concept that histamine might be a participating factor in the reaction. In 1910, Dale and Laidlaw[1] studied the pharmacologic action of histamine because it had been found as a constituent of ergot. They concluded their paper with the following statement: "Biedl and Kraus drew attention to the identity of the symptoms of

*From the Department of Pharmacology, Northwestern University Medical School, Chicago.

†Read before the First Annual Meeting of the American Academy of Allergy, New York, Dec. 12, 1944.

anaphylactic shock with those produced by intravenous injection of 'peptone,' which, as we have seen, are again very largely identical with those of B-I. The correspondence cannot yet be regarded as sufficient basis for theoretical speculation, and we content ourselves with recording, as a point of interest and possible significance, the fact that the immediate symptoms with which an animal responds to an injection of a normally inert protein, to which it has been sensitized, are to a large extent those of poisoning by B-iminazolylethylamine." This, as may be noted, is but the shadow of an accusing finger.

When Barger and Dale,[2] Abel and Kubota,[3] and others reported that histamine was a constituent of various animal tissues, the plausibility of histamine's involvement in anaphylactic reactions was considerably enhanced because it had by then become clear that the anaphylactic phenomena resulted from the interaction of antigen and antibody in the tissue cells and not in the circulating blood. There were reservations to be made with respect to this, however, because it was known since Ackermann's[4] early studies that various bacteria could decarboxylate histidine to form histamine, and these first tissue examinations did not exclude the possibility that histamine might be an adventitious substance formed post mortem. In 1927, however, Best, Dale, Dudley, and Thorpe[5] firmly established the fact that histamine is a normal constituent of many tissues. Further analyses have shown that it exists in almost all mammalian tissues, and especially in the so-called shock organs of the various species studied. Furthermore, it is there in adequate quantities to be significant in the event that it might be released. We do not know enough of the intimate metabolism of histidine and histamine to know how or why it is in these tissues, but that is another story. Analysis of the effects of histamine in various species of animals has extended the parallelism between these and the phenomena of anaphylaxis which Dale and Laidlaw first noted for the dog. These are imposingly extensive, well known to everyone, and need not be detailed here. There are a number of discrepancies between the reactions, or perhaps we should say apparent discrepancies, since a finer analysis has disposed of a sufficient number of them that we must view the disposal of the others as not improbable. The obvious fact that histamine does not duplicate in toto the anaphylactic phenomena is in no sense such a discrepancy. For example, we now know that heparin is liberated and can account for the incoagulability of the blood that is observed.[6] We do not expect it to account for the other phenomena and there is no reason to expect that histamine or anything else is necessarily the sole "anaphylotoxin." The anaphylactic problem is one of accounting for all the phenomena, no matter to what lengths this leads us.

The chief elements in the plausibility chapter of the indictment of histamine are then (1) that it can mimic many of the important an-

aphylactic reactions and (2) that it is present in the tissues where these reactions take place.

It was on this platform of plausibility and congruity that Lewis[7] framed his conclusions that the specific response of local anaphylaxis in the human skin represents a special form of cell injury leading to the release of H-substance and its specific effects. It was on this platform of plausibility and congruity that Dale[8] in 1929 phrased the more complete indictment of histamine in anaphylaxis as follows: "We may picture the anaphylactic shock, therefore, as the result of cellular injury, due to the intracellular reaction of the antigen with an aggregating antibody. Whether this is general or localized in a particular organ, histamine will be released and it effects will be prominent in the resulting reaction, imposing a general resemblance to the syndrome produced by histamine itself, on the symptoms seen in each species. The cell injury, however, is not limited to the degree required to produce a release of histamine, and involves other and more direct results. Such a conception is in accordance with all the facts as yet available, and it has the advantage of rendering intelligible, not only the striking resemblance between symptoms of the anaphylactic reaction and those produced by injecting histamine, but also the various and equally significant points of difference between the two syndromes."

As mentioned previously, this indictment was phrased by Dale on the basis of its plausibility and consistency alone. There was at that time no direct evidence available. It seemed incredible to me that such a brilliant investigator would not have attempted the verification of his hypothesis. I have subsequently learned in a personal communication from Dr. C. H. Kellaway of Melbourne, that he did perform some experiments which had some promising indications, but which he never reported.

The second chapter in the indictment of histamine consists of the experimental efforts to demonstrate an actual release of histamine in association with the anaphylactic reaction. The first important experiments in this category were those of Watanabe.[9] He demonstrated that there probably were differences in the histamine content of the dog's liver and the guinea pig's lungs before and after anaphylactic shock. I say "probably," because he did not examine the tissues of the same animal both before and after shock and because he knew there was a marked variation from animal to animal in the tissue content of histamine. He appreciated the temptation to conclude that his findings indicated a discharge of tissue histamine during the anaphylactic reaction, but for the reasons mentioned he did not do so. It is fair to say, however, that his observations contributed materially to the plausibility of histamine's involvement, even though they fall short of being unequivocal evidence. The next experiments designed to verify the histamine hypothesis and the first to succeed in showing

that histamine is liberated during an anaphylactic reaction in the intact animal were those of Dragstedt and Gebauer-Fuelnegg.[10] These investigators showed that the anaphylactic reaction in the dog was accompanied by the prompt appearance in the blood and thoracic duct lymph of a smooth muscle contracting substance having many of the characteristics of histamine. At virtually the same time, Bartosch, Feldberg, and Nagel[11] showed that perfusion of antigen through the isolated lungs of sensitized guinea pigs yielded a similar histamine-like smooth muscle contracting substance in the perfusate. Similar as well as modified forms of these experiments have been reported by now from many laboratories with confirmatory results. (The experiment as we have done it in the dog is so simple and easy to do, that I would recommend it to any who are called upon to teach or demonstrate anaphylaxis experiments. All that is required is to obtain about 10 c.c. of blood, heparinized, before the assaulting dose of antigen and again about two minutes after, centrifuge these blood samples and inject these plasma samples intravenously into an anesthetized and atropinized cat whose blood pressure is being recorded, or test them on the isolated intestinal strip of a guinea pig suspended in a saline bath.)

The questions then arise: Is this active substance histamine and, if so, is there enough of it so that one is justified in concluding that it is more than just an incidental by-product of the reaction? The active substance has been characterized as follows: it is a dialyzable substance of basic properties, stable to boiling with hydrochloric acid, inactivated by condensation with diazotized sulfanilic acid and by incubation with diamine oxidase or histaminase. It contracts the guinea pig intestine, and this effect is preventable by arginine; it lowers the blood pressure of the etherized atropinized cat and produces histamine-like wheals in human skin, etc. We can conclude that the active substance is most probably histamine because there is no other known substance in mammalian tissues or blood that meets these specifications. Attention should be called to the fact that this statement does not say that there are no other active principles liberated in the anaphylactic reaction.

If we are so sure that this active substance is histamine, why hasn't it been isolated in crystalline form so that the skeptic can be convinced by the data as to its melting point, crystal structure, etc.? The answer is as follows: The blood from a dog with a very severe shock will have a histamine activity of approximately 1 to 1.5 μg of histamine base per cubic centimeter. One can obtain from such a dog approximately 35 to 50 c.c. of blood per kilogram, by exhaustively bleeding him from the carotid artery. Thus a ten kilogram dog might yield from 350 to 500 c.c. of blood containing from 0.35 to 0.75 mg. of histamine. The chemical methods currently available do not permit the isolation and verification of crystalline material in such quantities. This consideration leads us to the other question—is the amount of histamine

liberated adequate to be of any significance in the reaction? This has been answered by two lines of evidence. Histamine is a freely diffusible substance, and Code[12] has shown that when histamine is injected intravenously its maximum concentration in the blood approximates that which one would expect, namely, that representing its more or less even distribution throughout the total body water of from 60 to 70 per cent of the body weight, e.g., the injection of 1 mg. per kilogram yields a concentration in the blood (and tissue fluids) of approximately 1.4 μg per cubic centimeter. More simply, one can determine the histamine concentration in the blood in a series of anaphylactic animals, and determine the corresponding amounts of histamine which one must inject in normal animals to achieve similar concentrations. This has been done and the results indicate that it would take approximately 1 to 1.5 mg. of histamine per kilogram to duplicate the blood concentrations found in fatal shock reactions and correspondingly lesser quantities to duplicate the blood concentrations found in shocks of lesser severity. Since the vascular reactions produced by the histamine injections are virtual replicas of the anaphylactic reactions in which the blood concentrations of histamine correspond, it seems there is no alternative, but to conclude that the histamine liberated in the dog is significant—and, more than that—substantially adequate to fully account for the vascular reaction of anaphylactic shock. To the statement that histamine is merely a concomitant elaboration from the tissues affected, it may be answered that it is impossible to have the amount of histamine free in the plasma of dog's blood that is found there in anaphylactic reactions without having histamine effects manifest in the animal. This statement will remain true until we have better histamine antagonists than those known at present.

The other line of evidence dealing with the amount of histamine liberated during the anaphylactic reaction comes from an examination of the liver histamine before and after shock. There is a great deal of evidence indicating that the liver is the "shock organ" in the dog—meaning that an hepatic reaction is extremely important in the genesis of the ensuing phenomena. We have shown that the liver histamine is abruptly decreased in an anaphylactic reaction, that the decrease is parallel to the severity of the reaction, and that the amount of the histamine decrease is consistent with the amount of histamine presumably liberated as calculated from the corresponding blood concentrations.[13] The significance of this agreement between two methods of quantitative evaluation cannot be disregarded.

There is a third chapter in the evidence against histamine which is as yet incomplete and unsatisfactory. This is the confirmation of its role in anaphylactic reactions which might come by inducing such reactions in animals which are protected from the effects of histamine. We have a number of substances which may be called histamine antagonists and a number of procedures designed specifically to pro-

tect an animal against histamine. There are none that are specific to histamine alone that are effective enough to make such experiments crucial and conclusive. The evidence from this line of investigation is consistent with the histamine hypothesis and will probably remain no more than this until we have better specific antagonists. It is appropriate to mention that the failure of a presumptive histamine antagonist to alleviate anaphylactic shock is of no moment until it is proved that it could antagonize histamine under the same conditions and in the same amounts as prevail in an anaphylactic reaction.

In the main, the evidence which I have cited bears on the relation of histamine to anaphylaxis in the dog. Personally, I have preferred to attempt to clinch the evidence in one case rather than accumulate suspicions in a variety of cases. Neither heparin nor histamine, nor the two together, account for all the phenomena of anaphylaxis in the dog. They do not account for the initial transitory hypercoagulability of the blood, for the leucopenia, for the changes in the sedimentation rate, etc. Histamine can account for the acute vascular reaction and for the acute lethal issue, and there is no other agent to which we can attribute these important phases of the reaction. Correspondingly, there is no evidence that the phenomena which are attributable to other things than histamine have vital significance.

The evidence for the role of histamine in the anaphylactic reactions of other animals is not quite so complete. Code's observations in guinea pigs indicate that it must play a substantial role in these animals. That it alone determines the issue of life or death we cannot say with finality, although there is considerable presumptive evidence that this may be so. For the rabbit we have definite evidence that histamine is released from the blood cells into the plasma during the reaction and that some may be released from the lungs. As yet the quantities released have not been adequately determined and we are unable to assess the magnitude of the effects attributable to histamine. There is reason to believe that leucocytic emboli occluding the finer vessels in the lungs may be an important contributing factor. In this connection it is important to remember that the character of the anaphylactic reaction varies in different species of animals and that we should expect that there would be some variation in the participation of various factors. For other animals the evidence is mainly that of the plausibility and consistency variety.

It is proper that we pay brief attention to the various challenges that have been made against the histamine hypothesis. The oldest of these arose from the observations of Lura and others[14] that sensitized guinea pigs which have become desensitized or anti-anaphylactic to the antigen do not show any desensitization to histamine and that an injection of histamine does not desensitize the animal to the antigen. Wells[15] long ago pointed out that we should not expect histamine to desensitize if it is the product of the antigen-antibody reaction, since

it is this reaction alone that is prevented by desensitization. I might add, that were it otherwise, we should expect desensitization to one antigen to convey complete desensitization to another antigen, for it is a well accepted tenet that the reactions, once produced, are identical irrespective of the antigen used. Heparin, as one of the by-products of the reaction, does not desensitize either to antigen or to itself, but no one seems to have mentioned this as a criticism or as a reason for denying its participation in the reaction. Smith's[16] report that quinine augments the susceptibility of sensitized animals to foreign protein but does not affect the reactions produced by histamine may be viewed in the same way. Many writers have referred to aspects of the anaphylactic reactions that are not accounted for by histamine. I know of no proponents of the histamine hypothesis that have maintained it as a universal doctrine. Dale's statement of the hypothesis was very explicit in this respect. Nor do I know of any reasons why we should view the anaphylactic reaction as admitting but a single operating factor. There is a certain amount of evidence, not yet very complete, indicating that other substances besides histamine and heparin may be concerned in some of the phenomena. For none of these do we have much evidence that they are very important in the reaction as a whole, although they serve to explain some minor phenomena. For example, while Schild[17] has shown that the addition of antigen to the bath, in which the uterus of a sensitized guinea pig is suspended, leads to the liberation of histamine from the uterus, he has also shown that the uterine contraction so produced is not wholly accounted for by the histamine released. Feldberg and Kellaway[18] and Kellaway and Trethewie[19] have presented evidence that another smooth muscle stimulating substance of a lysocythin-like nature may be partially concerned in this effect. A more pertinent discrepancy appears to exist in the observations of Rose and Weil[20] that the blood histamine of a rabbit decreases markedly and abruptly during anaphylactic shock and similar observations by Code and Hester[21] in the horse and calf. No one has suggested that these animals show withdrawal symptoms as the consequence of a sudden deprivation of histamine and I do not think that we have to resort to such an absurd and contradictory conclusion. For the rabbit, Abell and Schenck[22] have shown that during an anaphylactic reaction the leucocytes become sticky, adhering in clumps to the endothelium of the finer vessels, in many cases forming leucocytic emboli which obstruct them. Code[23] has shown that the leucocytes of rabbit's blood contain a very large amount of histamine, accounting for a substantial portion of the whole blood histamine, which is very high in this animal. Consequently, the leucopenic blood obtained by venous or arterial puncture during an anaphylactic reaction would be expected to show a decline in total histamine. Katz[24] has shown that the addition of antigen to the blood of a sensitized rabbit leads to the release of histamine from the blood cells. With this process going on

in the capillary beds where the leucocytes are confined, and predominantly so in the pulmonary circuit where the best opportunity for this exists, we have an ideal arrangement for eliciting the effects of histamine in a reacting organ. We have shown that the addition of antigen to the blood as it is perfused through the lungs of a sensitized rabbit results in both a leucopenia and a decreased histamine content in the recovered blood.[25] The histamine withdrawn from the blood in the lungs could not have been without effect there. In my opinion the analysis of the contradictory findings in the rabbit has served but to strengthen the histamine hypothesis. No similar studies have been made in the horse and calf, but we have a pattern by which we may view the findings in these animals as not necessarily precluding the participation of histamine in them.

Finally, there are those who view the histamine hypothesis as an alternative or conflicting theory to an antigen-antibody hypothesis. This is not the case as Dale visualized it, nor is there any good reason for such a view. Viewed as but one of the consequences of the antigen-antibody reaction, the tissue release of histamine renders intelligible many of the reactions that occur. We do not know the mechanism by which this release is brought about, nor do we know for all cases whether this release is of minor or major importance. In the cases that have been studied most thoroughly it seems to be of major importance but this is no reason to assume that it must be of equal consequence in all cases. This knowledge of the identity of an adversary, coupled with an appraisal of his harmfulness should be of considerable value in formulating an intelligent defense.

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NUTRITIONAL ASPECTS OF GLOBULIN METABOLISM*

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RECENT progress in the field of immunochemistry has made the immunologic significance of globulin metabolism more evident. As Marrack[1] recently said, "Now . . . that a large volume of evidence has accumulated which shows that antibodies are modified serum globulins, the chief problem has become the structure and properties of proteins in general and the special characteristics of antibodies in particular." Therefore, if we are to understand the deeper significance of antibodies and the antibody mechanism, we must learn more about these special characteristics; and, moreover, if antibodies are plasma globulins, they must be studied in relation to the globulin-producing tissues.

Granting that antibodies are specifically modified plasma globulins, that fact by itself is not enough, because "globulin" is not a homogeneous substance but consists of at least four chemically distinct fractions, namely, fibrinogen, alpha, beta, and gamma globulin. Furthermore, Tiselius and Kabat[2] have demonstrated by electrophoretic analysis that most antibodies occur in the gamma fraction of immune plasma globulin or in fractions related closely to it. More recently Enders and his collaborators[3] have demonstrated specific antibodies for sixteen different infectious agents in human gamma globulin. Of interest, also, are the experiments of Newell et al.[4] which showed the presence of skin-sensitizing antibody in the gamma fraction of serums from allergic individuals. Thus, it is obvious that particular attention must be paid to gamma globulin if we are to learn about the fundamental nature of antibodies and, especially, about their chemical composition, mode of origin, and manner of formation. Answers to these questions will undoubtedly bring us nearer to the solution of many problems of infection and resistance, and, possibly, of allergy as well.

The chemical complexity of gamma globulin has been revealed recently by Brand, Kassell, and Saidel[5] in their studies of the amino acid composition of human plasma proteins. For gamma globulin they found the following amino acid percentages by weight: arginine, 4.80; histidine, 2.5; lysine, 6.7; cysteine, 0.70; half-cystine, 6.75; methionine, 1.06; tryptophan, 2.86; tyrosine, 6.75; serine, 11.4; threonine, 8.36; and leucine, 9.32. It is especially significant that, as evidenced by this analysis, human gamma globulin contains at least five of the eight amino acids found recently by Rose and his collaborators[6] to be essential in man for the maintenance of nitrogen balance. The conclusion seems warranted, therefore, that the synthesis of such a complex pro-

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tein must require an abundant assortment of these essential amino acids within the tissues; these amino acids are available either in the protein reserves or as dietary protein.

Substantiation of this evidence of the chemical complexity of gamma globulin has been found also by the use of a method of biologic assay whereby proteins of varying nutritive quality are fed to hypoproteinemic adult white rats for a period of from five to seven days, and the effects are determined by means of differences in degrees of weight recovery and by increased or decreased concentrations of total serum protein.[7] For example, when a protein-depleted rat ingests an adequate quantity of a high-quality protein as its principal source of dietary nitrogen, there results a rapid weight recovery and an increased concentration of total serum protein; in contrast, when such an animal ingests a similar quantity of a protein of low biologic value there follows but little weight recovery or increase in concentration of total serum protein. It should be emphasized that all rations are made isocaloric and the basal ration to which the test proteins are added is adequate with respect to its vitamin and mineral contents. When plasma protein, composed predominantly of gamma globulin and derived from either human or bovine source, is fed to such protein-depleted rats, the response is essentially identical with that observed when similar quantities of a high-quality protein are eaten. We conclude, therefore, that these two types of plasma protein are also high-quality proteins and that, as the chemical analysis has indicated, their nutritive value is due, largely, to their varied assortment of amino acids essential for the maintenance of an effective nitrogen metabolism in the rat.

Although all are agreed that the formation of plasma albumin is dependent upon dietary protein intake, there is still uncertainty concerning the influence of diet on the synthesis of plasma globulin. This uncertainty is due, presumably, to the fact that, in the milder grades of protein inadequacy, the fall in concentration of plasma albumin tends to overshadow that of plasma globulin. It may be, also, that in the presence of a developing hypoalbuminemia there is a compensatory contribution of globulin to the blood plasma. At any rate, the answer to the question is probably related to the method employed for studying it. Whipple and his associates[8] have maintained that "as measured by plasmapheresis, diet regulates globulin production equally as well as albumin." We have found[9] in markedly hypoproteinemic rats, a diminution in the absolute amounts of plasma protein, not only of serum albumin but of serum globulin as well, and of all three globulin fractions precipitated by varying concentrations of sodium sulfate, viz., euglobulin and pseudoglobulins I and II.

Further evidence of the influence of amino acid intake upon the production of plasma globulin is furnished by experiments[10] demonstrating that the feeding of the ten "growth" amino acids (Rose) to

hypoproteinemic dogs, in adequate amounts, engenders satisfactory rates of plasma protein regeneration, including both plasma albumin and plasma globulin. In addition, the direct participation of dietary amino acids in the construction of plasma globulin has been shown by Schoenheimer and his associates,[11] using isotopic amino acids. By this technique they were able to demonstrate that the utilization of isotopic amino acids is approximately the same for all fractions of the plasma protein, including fibrinogen, euglobulin, pseudoglobulin, and albumin, and that, when isotopic amino acids are fed to actively immune rabbits and rats, "antibody, like the other serum and body proteins, participates in metabolic reactions involving the uptake of dietary nitrogen." Of particular interest is the observation that this interchange does not occur in relation to antibody globulin introduced passively. All of the above evidence surely justifies the conclusion that amino acids enter directly into the process of synthesis of plasma globulin. The further implication is that the fabrication of antibody globulin is a part of the general process of protein metabolism and that, for this reason, acquired resistance depends, in last analysis, upon the processes of nutrition.

Under ordinary conditions of good nutrition, there is a sufficient intake of dietary protein to enable the body to fabricate plasma proteins at a rate commensurate with its daily needs. Further, there is always available in the tissues a reserve of protein material, the so-called deposit protein, or protein reserves. When, however, because of prolonged periods of starvation, these protein reserves become depleted, there results a loss in the capacity of the individual to synthesize specific antibody. We have demonstrated this in rabbits and white rats for such types of antibodies as agglutinins, precipitins and hemolysins.[9, 12] In other words, in the absence of an adequate supply of essential amino acids (obtainable either from the tissue-protein stores or from the daily ration) during immunization, specific antibody cannot be fabricated normally. This fact suggests the probability that, whenever an animal with a pronounced protein deficiency becomes infected, its ability to acquire resistance quickly or to mobilize a specific immune mechanism effectively will likewise be impaired. This same effect may be manifested in the human being with a debilitating disease, such as chronic nephrosis, where prolonged proteinuria and exhaustion of the protein reserves may lessen the capacity to react to a spontaneous pneumococcic infection. It should be pointed out, also, that antibody globulins from the human being, horse, and rabbit are therapeutically interchangeable, thus suggesting an essential similarity in their chemical composition, at least with respect to their requirements for amino acids in the process of synthesis.

Just as protein depletion leads to a lessened capacity to synthesize specific antibody, so does the refeeding of good quality proteins tend to restore the normal ability to fabricate antibodies. Thus, when rats

which are markedly hypoproteinemic are fed high-quality proteins, such as dehydrated beef or lactalbumin, for several days and are then subjected to antigenic stimulation, they quickly regain their capacity to elaborate specific antibody; in fact, they may almost equal the antibody output of the control animals. This they do not do, however, if markedly incomplete proteins, such as gelatin, are fed as the principal source of dietary nitrogen during the period of repletion. Consequently, we assume that, in an otherwise healthy animal, repletion of the protein pools by ingestion of proteins furnishing a proper assortment of all the amino acids which go into the antibody molecule, will enable the animal to fabricate specific antibodies almost as well as a normal animal.

Besides these theoretical problems, there are practical ones related to them which should be mentioned. One is the problem of the role of amino acids, either synthetic preparations or protein hydrolysates, in the practice of allergy. The former have been utilized recently by Olmsted et al.,[13] whereas the latter have been used by several workers, including Hill.[14] It has been shown that, with adequate amounts of these preparations, a human being can be kept in nitrogen balance on a nonprotein diet. Another use for them is for the maintenance of a patient in nitrogen balance preparatory to testing for hypersensitiveness to some particular food protein. In either use of the hydrolysates, however, it is essential that they be (a) nonantigenic and (b) complete with respect to their content (in adequate amounts) of the eight amino acids essential for man, viz., tryptophane, methionine, leucine and isoleucine, phenylalanine, valine, threonine, and lysine. At the present time these hydrolysates are prepared from various protein sources, such as casein, lactalbumin, fibrin, fish muscle, and various protein mixtures, either by enzymatic, or acid, hydrolysis. Tryptophane is destroyed, though, when the latter method is employed, and must be added later.

We have tested several of these preparations, utilizing our assay method of feeding the digests to the hypoproteinemic rat as its principal source of nitrogen and have found considerable variability in their nutritive potentialities. Of those which we have thus far tested, the enzymatic digests of casein have, in general, given the best results. The acid digests, on the other hand, have been definitely less nutritive, due, presumably, to their inadequacy of essential amino acids, probably mostly tryptophane. An interesting fact in this connection is that, in our experience, a starving, but otherwise healthy, hypoproteinemic rat will always eat a ration which contains high-quality protein as the main source of dietary nitrogen, whereas, if a protein of lower biologic quality in the same proportions is fed, he will eat much less of his daily ration. This seems to apply also to the feeding of the protein hydrolysates; accordingly, there is reason to believe that, if a rat will not eat these mixtures, the other dietary essentials (vitamins

and minerals) being present, such a mixture is probably lacking in adequate amounts of some one or more of the essential amino acids necessary for the well-being of the rat.

It is obviously important, in the clinical use of these digests, that they shall have been adequately tested and shown to contain *all* of the essential amino acids in *adequate* amounts. In other words, it is necessary for each company making the digests to demonstrate by rat growth, nitrogen balance, or other tests that their product is complete in essential amino acids, and that it may be able, therefore, to maintain a patient in nitrogen balance when utilized as the sole source of dietary nitrogen.

This brief discussion has dealt largely with the chemical make-up of plasma globulin and antibody-globulin and has not been directed at the underlying problem of globulin synthesis. Enough evidence is available at the present time to indicate that gamma globulin in man contains many, and possibly all, of the amino acids essential for the maintenance of nitrogen balance. Evidence as yet is lacking, though, concerning the mechanisms of globulin synthesis by intracellular enzymes or vitamins. Undoubtedly, in this latter field much will be learned, in time, about the deeper significance of antibodies and the antibody mechanism.

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TREATMENT OF URTICARIA WITH SYNTHETIC VITAMIN K*†

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IN SEPTEMBER, 1941, Howell[1] stated: "Prothrombin and fibrinogen are constantly being formed and consumed in the blood probably forming fibrin which may undergo proteolysis and provide nutrition for cells or may protect the integrity of the capillary walls." That prothrombin formation might have something to do with the permeability of the capillary wall and that this mechanism might be affected by the administration of vitamin K led to this investigation.

From September, 1941, to September, 1944, we have seen 305 cases of what we term chronic urticaria. Of these, 14 per cent could be designated as angioneurotic edema, and 68 per cent as urticaria; 18 per cent showed lesions of both types. We believe the conditions are of common origin and pathology and have considered them as identical in this study.

The usual methods of investigation were used, including skin tests, both scratch and intradermal, trial diets, search for infections, and avoidance of drugs, with relief obtained in 149, or 49.5 per cent. The remaining 156, or 50.5 per cent, which were not relieved by these methods were then given vitamin K.

Of these 156 patients, 29 per cent were males and 71 per cent were females. One was 4 years of age, one 6, one 11, one 12, and five were between 13 and 19 years of age. The others were young and middle aged adults.

The duration of the urticaria varied from one month to twenty years; the number and the distribution of the cases according to duration is shown in Table I. None of the cases of less than one month's duration was included in this study, and all were considered chronic.

The frequency of attacks varied from constant to irregular and infrequent with 110 patients showing some lesions every day. In 8 per cent of the entire group other allergic manifestations were present.

The material used was Menadione (2-methyl-1,4-naphthoquinone), an analogue of vitamin K, with the trade name of Kappaxin. All administration was oral.

At the time this study was begun there was some fear of the ill effects of vitamin K, consequently the first patients treated received small amounts with poor results. With increased dosage the results improved. Two milligrams of vitamin K daily failed to relieve any of six patients of whom five were relieved later by 6 mg. daily. Three

The material used was generously supplied by the Medical Research Department of the Winthrop Chemical Company.

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TABLE I

DURATION OF URTICARIA

DURATION	NUMBER OF PATIENTS	DURATION	NUMBER OF PATIENTS
1 month	22	3 years	12
2 months	26	4 to 6 years	14
3 months	8	7 to 11 years	3
4 to 6 months	15	13 years	3
7 to 11 months	13	15 years	6
1 year	22	17 years	2
2 years	8	20 years	2

milligrams daily failed in four patients of whom two were relieved by 6 mg. daily. Four milligrams daily failed to relieve seven patients, but was adequate in eight. Six milligrams daily failed in 45 patients and relieved seventy-four, while 9 mg. daily, given to twelve patients, failed in four and relieved eight. Six milligrams then was adopted as the routine daily dosage and 2 mg. were given before each meal. Bile salts were used by approximately one-half the patients, four of whom were already using bile salts because of some biliary disturbance. We were unable to observe any difference in results due to the bile salts.

Prothrombin time determinations were done using the method of Smith and associates.[2] Fresh blood was drawn from the vein without a tourniquet using a 26 gauge needle. The blood was immediately mixed in a tube with thromboplastin, and the clotting time was determined. Twenty-five to 30 seconds was considered normal time and, arbitrarily, we took 35 seconds as the upper normal limit. If 30 seconds had been made the top normal time, it would have added one cure and one failure to the group with prolonged time.

In the beginning, thirty-seven patients were treated without prothrombin time determinations. Then 119 were treated after prothrombin time determinations. Table II shows the results obtained.

Aside from the fact that 78 per cent of those with prolonged times were relieved, there are two very interesting facts shown in this table. One is that 32.5 per cent of those with normal prothrombin times were relieved; the other is that of the group in which no time determinations were done, 43 per cent were relieved by treatment. If we should assume that the proportion of those with normal and prolonged times is the same in this group as in the group whose times were determined, we should expect the percentage relieved to be 62. Whether

TABLE II

RESULTS OF TREATMENT

PROTHROMBIN TIME	NUMBER OF PATIENTS	NUMBER RELIEVED	PERCENTAGE RELIEVED
Not done	37	16	43.0
Normal	41	13	32.5
Prolonged	78	61	78.0
Controls (normal time)	56	0	0.0

TABLE III

CORRELATION OF PROTHROMBIN TIME WITH PERCENTAGE RELIEVED

TIME (SEC.)	PERCENTAGE RELIEVED									
	10	20	30	40	50	60	70	80	90	100
30 to 35			×							
36 to 40					×					
40 to 45						×				
46 to 50						×				
50 to 55										
56 to 60										×
60 to 65										×

this disparity is due to the small number in the group or to some other and unknown factor we cannot say.

As controls, fifty-six persons suffering from asthma, allergic rhinitis, and allergic eczema were treated. No relief was obtained in any case. All of these had normal prothrombin times.

The correlation between the prothrombin time and the percentage of patients relieved is shown in Table III.

The duration of treatment varied from one to four weeks. In many instances lesions failed to appear after two days of treatment. Of those relieved, 75 per cent were free from lesions within one week. Four patients required eighteen to twenty days to clear up.

Of the ninety patients relieved by treatment, twenty-eight, or 31 per cent, had a relapse. These relapses occurred following periods of freedom varying from two weeks to eight months. Readministration of the vitamin brought about prompt relief in each instance. We have not yet seen a second recurrence in any patient.

Repeated prothrombin time determinations show a drop to normal in those with prolonged times if the treatment is adequate. A drop from 50 to 25 or 30 seconds within forty-eight hours was quite common. How much sooner than this the drop may have occurred we do not know, for difficulties of private practice made more frequent determinations impracticable. Those whose time was prolonged, but in whom treatment was unsuccessful, showed the same drop in time as those who were satisfactorily relieved.

DISCUSSION

It appears that in approximately one-half of the patients with chronic urticaria coming under our observation, the cause was not found by the usual diagnostic methods. Of this group, approximately two-thirds had prolonged prothrombin times, and approximately four-fifths of these responded satisfactorily to treatment with vitamin K. Even in the group not showing prolonged prothrombin time, nearly one-third are relieved by treatment. Of the entire group of what we would have classed as failures, 62 per cent were relieved by vitamin K.

It is interesting that 32.5 per cent of those with normal time were relieved by treatment. It could not be that the normal has been set

too high, for all fifty-six controls had times within the limits set as normal, and no patient was seen whose time was lower than 26 seconds.

It is interesting, too, that treatment reduced to normal the prolonged time in seventeen patients without giving relief from symptoms. Certainly the prolongation of prothrombin time is important for the difference in the number relieved among those showing normal times and those showing prolonged times is as 32.5 per cent to 78 per cent which is enough to be significant. But it may well be that difference in the prothrombin content of the blood is only part of the explanation of these cases of urticaria, and that other factors may be involved. Then, too, it is always well to keep in mind the erratic behavior of urticaria and draw conclusions with a great deal of caution from small samples.

CONCLUSIONS

1. Approximately one-half of the patients observed to have urticaria were not benefited after the usual methods of diagnosis and treatment.

2. Of those cases in which the usual methods failed, approximately 62 per cent were relieved after administration of synthetic vitamin K.

3. The prothrombin time is of much help as a prognostic sign, since 78 per cent of those with prolonged time were relieved as compared with 32.5 per cent of those with normal time.

4. This method of examination and treatment may be of some help in classifying patients with urticaria etiologically.

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MOISTURE CHARACTERISTICS OF POLLEN*†

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INTRODUCTION

VERY few data are available in modern literature dealing with the moisture content of pollen or the preservation of pollen for storage. Since it is known that pollen extract deteriorates quite rapidly even at refrigerator temperature,[1] it is reasonable to assume that residual moisture in pollen stored at room temperature may be responsible for a considerable loss of antigenicity. Likewise, every specimen of pollen contains mold spores, but their number is usually negligible. However, if the pollen is wet so that spores can grow, the contamination may become sufficiently great to alter the specific activity of the extract. From a purely economical standpoint, the greater the water content of the pollen, the higher is the price paid for its antigenic material under present methods of selling pollen by weight. In view of these facts, it seemed worth while to investigate the water content of various commercial pollens to see if some practical method could be devised for their storage which would give reasonable assurance of maximum potency at the time they are used for making extract.

METHODS

Two methods of drying pollen were used in this work, the pollen dehydrator[2] which was developed in our laboratory, and the Cryochem apparatus. It was found with the latter that samples of pollen no longer lost moisture after eighteen hours of high-vacuum drying, and all samples were dried for at least this length of time. It was questionable whether or not high-vacuum drying would dry pollen to completeness, and, to determine this, a sample of short ragweed pollen, which had been dried by high vacuum, was exposed to a temperature of 65° C. in the pollen dehydrator for four hours. It lost an additional 0.3 per cent moisture. A similarly vacuum-dried specimen was heated in a gas oven to 100° C. for one-half hour. It lost an additional 1 per cent moisture. At this temperature, however, there is a strong possibility that other volatile materials than water vapor were lost by the specimen. The 1 per cent loss represents the maximum possible residual water, but the actual water remaining after high-vacuum drying is probably less than that figure. It is seen, therefore, that high-vacuum drying removes virtually all the water from pollen, and, since it is a method which can

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in no way injure the antigenic substance of the pollen, we propose that high-vacuum drying be used as a standard in determining and specifying the moisture content of antigenic materials.

WATER CONTENT OF COMMERCIAL POLLENS

The first modern analysis of the water content of pollen is that of Heyl[3] in 1917, who proved his self-collected specimen of short ragweed pollen to contain 5.3 per cent moisture. A similar value was reported by Koessler[4] in the following year. Since then we have found no further figures, but Durham[5] points out that the specific gravity of pollen is closely dependent upon atmospheric relative humidity.

During the pollen collection seasons of 1943 and 1944, the moisture content of twenty-five different specimens of pollen was determined by measuring their loss in weight when subjected to high-vacuum drying on the Cryochem apparatus. The results are tabulated in Table I.

TABLE I

COMMERCIAL POLLENS

MOISTURE CONTENT IN PER CENT			
Spring Pollens		*Early Summer Pollens*	
Alder	7.1	Timothy	9.8
Elm	5.4	Timothy	5.7
Hazel	4.6	English plantain	10.5
White ash	19.3	English plantain	8.7
Red oak	11.4	English plantain	10.9
Mulberry	5.3		
Average	8.9	Average	9.1
Fall Pollens			
Giant ragweed	7.6	Short ragweed	11.2
Giant ragweed	8.6	Short ragweed	6.5
Short ragweed	8.0	Chenopodium	9.3
Short ragweed	6.9	Mugwort	6.2
Short ragweed	7.0	Marsh elder	4.8
Short ragweed	6.4	Cocklebur	5.1
Short ragweed	4.8	Cocklebur	6.1
Average 7.0			
Grand average 7.9			

It will be noted that there is a wide variation in moisture content; the extremes were 4.6 per cent and 19.3 per cent, and the average of all twenty-five was 7.9 per cent. When these averages are broken down for the various seasons, however, it is seen that the spring and early summer pollens average 8.9 per cent and 9.1 per cent, respectively, whereas the fall pollens average 7 per cent. This is a decrease of 2 per cent on an absolute scale, but actually means that the fall pollens contain, on an average, 22 per cent less water than the spring and early summer pollens. This is accounted for by the higher humidity which usually obtains in this part of the country during the early collection seasons.

To investigate further the hygroscopic nature of pollen, two samples of short ragweed pollen from the same collection were used. One was defatted with anhydrous ethyl ether, one was left normal. Both were dried by high vacuum, placed on watch crystals, and exposed to air. They were weighed from time to time over a period of forty-eight hours. The initial weighing could not be done with accuracy because the absorption of moisture was so rapid as to preclude weighing on an analytic balance. However, it was found that no more moisture was absorbed after three hours, and forty-eight hours later there was actually a decrease in weight due to a coincident drop in atmospheric relative humidity. Both samples absorbed approximately the same amount of water, proving that those fractions of the pollen that are soluble in ether are not the ones primarily concerned with the hygroscopic nature of the pollen.

COMMERCIAL DRYING PRACTICES

Little has been written on the subject of drying pollen, though all authors are unanimous in the opinion that pollen must be dry if it is to be stored safely. Kelly[6] recommends heating the pollen "not above 75° C. for 24 hours" or exposing it several hours to sunlight, then placing it in a dessicator over sulfuric acid "until all traces of moisture have been removed." He does not, however, tell how one knows when all the moisture has been removed, and, since a temperature of 75° C. is sufficient to cause some scorching of the pollen, this would seem an unwise procedure. In view of the well-known fact that ultraviolet light causes profound changes in proteins, direct sunlight exposure cannot be recommended, and some measure should be taken to filter out the ultraviolet rays.

Most pollen collectors simply expose their pollens to air in a thin layer for twelve to forty-eight hours or until it "looks dry." Some then bottle them; others keep them in dessicators over calcium chloride, silica gel, or some other drying agent. There is no set rule. Each does as his conscience and facilities dictate, and the final product depends on these factors as well as the highly variable one of the atmospheric relative humidity.

It is a well-known fact that the antigenic content of pollen is highly variable with time, locale, and year of collection. Unquestionably, genetic factors in the strains, the fertility of the soil, and the season's precipitation greatly affect the nitrogen content of the pollen. These factors have not yet been worked out, though they are being given consideration. But it is also likely that much of the antigenic substance that is in the pollen is needlessly sacrificed by careless or inadequate handling at the time of collection.

PRACTICAL DRYING

It is our belief that every pollen collector should have some method of quickly and safely drying pollen immediately on collection. It does

not have to be elaborate or expensive, but it does have to do a thorough job. We have described such a machine,[2] but there are any number of similar possibilities as well as the high-vacuum apparatus.

There are two simple observations that will tell if the collected pollen is dry. When dry pollen is being transferred to a container from the glazed paper on which it had been placed in the pollen dehydrator, much of it will stick to the paper. If this is scraped with a wooden spatula, it will be found that the grains take on strong electrostatic charges which cause them to jump up from the paper, requiring great care to prevent loss. A pollen containing more than 2 per cent water will not do this.

Second, in our recent analyses when dry pollens were used, it was discovered that the weight loss after ether extraction was much less. It was almost nil or amounted to approximately 2 per cent. With commercial pollens a weight shrinkage of 5 to 10 per cent was always expected from defatting. In other words, a large part of the loss which was formerly attributed to the ether soluble substances extracted from the pollen was due primarily to dehydration accomplished by anhydrous ether. It is interesting to speculate on how much water soluble substance has been lost in the removal of this water by ether.

HANDLING OF POLLEN FOR STORAGE

Our experience has resulted in a system for the handling of pollen which we feel guarantees the maximum of antigenic material in pollens which are to be stored for indefinite periods.

The first step is immediate drying on collection in a pollen dehydrator by the collector. The vagaries of the atmosphere make air drying too haphazard. After machine drying, the pollen should be sealed in dry, airtight containers and sent at once to the laboratory. Upon receipt at the laboratory, the pollen is promptly defatted with anhydrous ethyl ether in a Soxhlet apparatus which is protected against atmospheric moisture by a drying tube containing calcium chloride, silica gel, or similar agent. Finally, the pollen is placed in a rubber-stoppered bottle. A 20 gauge hypodermic needle is thrust through the stopper and connected with the Cryochem machine for twelve hours. Then, while the suction is still in operation, the needle is withdrawn and the whole top of the bottle plunged immediately into melted paraffin. The pollen is then ready for storage. Samples opened several months after such handling have all shown the vacuum to have remained apparently undiminished.

SUMMARY

1. High-vacuum drying of pollen removes moisture to virtual completeness. It is proposed that this method of drying be adopted as the standard on which to base estimates of water content.

2. Samples of commercial pollens were found to contain from 4.6 to 19.3 per cent moisture, when subjected to high-vacuum drying.

3. The moisture content of pollen closely follows atmospheric relative humidity.

4. The hygroscopic nature of pollen is not primarily concerned with its ether soluble fractions.

5. A method of preparing pollen for storage is presented.

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STUDIES IN FOOD ALLERGY

IV. Variations in Allergenic Activity of Food Extracts*†

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SINCE their introduction into clinical use about thirty years ago, allergenic extracts have found extensive clinical application in the diagnosis of allergic conditions. The extracts prepared at first were rather crude and limited in number, and, because the pioneer workers had to determine the proper testing strength entirely by experience upon individual patients, irritating nonspecific positive skin reactions were frequent. Likewise the trial and error procedure, especially with a new extract, induced at times alarming constitutional reactions particularly after intracutaneous testing. This was obviated to a large extent by subsequent additional experience gained through more extensive use of the extracts. It served to determine that concentration of extract which would give a specific positive reaction in a sensitive patient without inducing constitutional reaction upon intracutaneous testing or irritative reactions in nonallergic controls. The use of a preliminary scratch test with a new unknown extract served to act as a guide for the proper subsequent dilution to be used in routine testing.

The introduction of the nitrogen method of determination based upon the belief that the active excitant of allergens was protein in character seemed at the time to be the solution to the problem of extract standardization. Although it later was shown that nitrogen determination did not necessarily represent a true measure of the allergenic excitant, in the hands of many workers, determination of nitrogen content, whether total or protein nitrogen, provided a satisfactory means of standardization especially for pollens and inhalants.

Extensive clinical experience and subsequent refinements in the technique of preparation of extracts and of testing methods gradually taught allergists what seemed to be the proper and safe concentration of various allergenic extracts to be used for routine testing regardless of the method of standardization employed. The ideal concentration was considered as one which provoked a specific positive reaction in a hypersensitive individual without inducing a constitutional reaction. It soon was discovered that extracts containing potent allergens could be diluted sufficiently to avoid constitutional reactions and yet induce highly specific positive skin reactions; on the other hand, extracts containing weak

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allergens had to be used in such high concentrations that nonspecific irritative reactions were common.

At the present time, therefore, allergenic extracts employed particularly for intracutaneous testing are standardized either by simple dilution or by the determination of the total or protein nitrogen content. The concentration employed in different clinics varies according to the method used and the special experience of the individual clinic. Substances like pollens, certain epidermals, or such foods as egg white or milk, which contain large amounts of easily extractable excitant, provide extracts that are specific and fairly uniform. They are easily standardized so that the same concentrations can be employed for testing whenever a new lot is prepared. On the other hand, the extracts of substances like fruits and vegetables contain small and extremely variable amounts of active allergenic excitant and larger quantities of extraneous material; they thus provoke a larger percentage of false positives. Such extracts are much more difficult to standardize and are subject to considerable variation in newly prepared lots of extracts. Consequently, they often are standardized by what might be called the trial and error method—that is when a new lot of extract is prepared, it is used in a concentration which previously has been employed. If further experience with this extract indicates that it induces too many nonspecific positive reactions, then the extract is diluted further to overcome this difficulty; if too few positives are obtained, the concentration sometimes is increased. Such a procedure obviously is crude and productive of many false positives and negatives before the correction is made. This brings about not only a variation in the percentage of positives of different lots but also a variation in the reports from various clinics as to the degree of sensitivity to certain allergens.

These facts are familiar in a general way to most of us; however, little actual discussion of the problem or reports of comparative studies are found either in textbooks or the allergy literature. It happens that we have had an opportunity in our clinic at Temple University Hospital to make a comparative study of the methods of standardization as applied to all extracts and particularly to foods. The results of this study provided data which seemed to be sufficiently interesting to warrant their report in the present communication.

PROCEDURE

The *material* for this study was accumulated in two five-year periods between 1933 and 1943 and a third period since 1943. During the first period between 1933 and 1938 all the food extracts used for routine skin testing were standardized by simple dilution. The concentration used depended upon previous experience with similar extracts in our own clinic. Total nitrogen values were ascertained beforehand on all these extracts so that a comparison between the two methods of standardization was possible.

At the end of the first period we reviewed the data which had accumulated and decided from this review to attempt to employ the concentration in the total nitrogen method which seemed to be best for testing purposes. All extracts were standardized by this method and used in the concentrations thus determined.

During the second period between 1938 and 1943, therefore, we had a period of five years in which the results of tests could be analyzed and compared with the period during which the simple dilution method was used. All through both periods, extracts of the inhalant allergens were standardized for routine testing solely by total nitrogen, which thus provided a means of comparing the inhalant and ingestant extracts. Where the inhalant allergen showed many false positives at the end of the first period, a change in its concentration was made during the second testing period.

At the end of the second period, after reviewing the results of our ten-year survey, it was decided to standardize the extracts by what we termed the method of *biologic assay*. This is based on a similar principle used in such serologic procedures as the typhoid agglutination test where the titrations obtained on normal individuals are considered as within normal limits and anything above it as positive. We accordingly tested all new extracts in varying dilutions directly on nonallergic individuals and, when available, on patients known to be sensitive to these allergens. The final testing concentration was that which failed to produce nonspecific reactions in the controls and yet evoked specific reactions in the hypersensitive patient. This procedure was adopted for each lot of extract prepared and the results recorded for future comparison.

Finally, in order to obtain some information as to the possibility of applying this method of biologic assay for standardization we obtained twelve commonly used food extracts from four other clinics and subjected them to a comparative study with our own extracts. All the extracts were employed in the concentration used by each clinic for routine testing. Each extract was tested upon a group of nonallergic controls and, whenever possible, also upon all allergic individuals including those who previously gave positive reactions in our own clinic. All tests and readings were done by one individual for the sake of uniformity, and the findings recorded and compared.

RESULTS

One of the first things to attract attention in the analysis of our results was the wide variation in the total nitrogen values of certain food extracts even though prepared under standard conditions by the same technician. The original material from which all extracts were prepared was always of the highest available quality, and attempts were made with each new lot to use as nearly constant and uniform material as feasible. This was possible when extracting foods containing large

TABLE I

FOOD	1	2	3	4	5
Banana	.64	.045	.25	–	–
Grapefruit	.63	.38	.63	.373	–
White Potato	2.23	.41	4.0	1.9	–
Lettuce	.605	1.16	–	–	–
Orange	1.24	1.449	2.3	–	–
Peach	.31	.62	.31	–	–
Carrot	.10	.91	.94	2.0	.872
Strawberry	.165	.535	.7	.95	–

amounts of easily extractable excitant, for example, meats, fish, nuts, or cereals. Consequently these foods showed fairly uniform skin reactions when different lots were used. By contrast, most fruits and vegetables contain relatively smaller amounts of excitant and more extraneous material. It is more difficult, therefore, to keep the original material constant when preparing extracts. Hence, as indicated in Table I, this group showed marked variation in nitrogen values in different lots even though all were prepared exactly in the same manner.

For the purposes of this presentation we have chosen those members of the group which showed the variation to the greatest extent and for which we have satisfactory figures for several lots. It was noted, however, that similar variation existed among many of the fruits and vegetables, so that anything said about those included in Table I can be considered as also applying to the others.

If the nitrogen values were a true index of the allergenic activity of these extracts, some correlation should exist between the nitrogen content of the extract and the percentage of positive skin reactions. Table II presents an analysis of this data, using the same fruits and vegetables as shown in Table I. During the 1933 to 1938 period, the extracts of these foods were standardized for testing by simple dilution, while fixed nitrogen values were used for the 1938 to 1943 period. These latter values were arrived at by experience gained from the 1933 to 1938 survey. The final concentration chosen usually represented the mean of all extracts used, making allowances for excessively high and low percentages of positive reactions.

It will be noted that during the second testing period, when the skin testing concentration of the extracts was changed, a decrease in the nitrogen values was accompanied by a corresponding decrease in the percentage of white potato, grapefruit, and banana but by an increase in the percentage of lettuce, orange, and peach. However, an increase in the nitrogen content resulted in a decrease in the percentage of reactions with carrot extract and no change with the strawberry extract. One might infer with some justification from these findings that standardization of such extracts by nitrogen content is not entirely reliable. However, this probably is more a criticism of the source from which the extract is derived than of the method. This is supported by our results with the inhalants and certain of the food allergens. Thus,

TABLE II

FOOD	1933 TO 1938						1938 TO 1943					
	CONCENTRATION	TOTAL TESTED	PERCENTAGE POSITIVE				CONCENTRATION	TOTAL TESTED	PERCENTAGE POSITIVE			
			+	++	+++				+	++	+++	
Banana	1:5 .128	408	68	13	1	20.1	.05	337	43	1	1	13.3
Grapefruit	1:5 .10	219	27	1	0	12.8	.05	337	19	2	0	6.2
White potato	1:5 .384	316	47	9	1	14.8	.2	337	42	3	0	12.5
Lettuce	1:5 .121	442	24	3	0	6.3	.1	354	28	4	0	9.3
Orange	1:5 .228	447	24	1	0	5.6	.1	141	17	0	0	12.1
Peach	1:5 .062	433	22	4	1	6.2	.05	348	36	4	0	11.5
Carrot	1:5 .02	404	79	2	2	20.5	.05	167	13	2	0	8.9
Strawberry	1:5 .033	425	56	9	2	13.5	.05	348	54	2	1	13.5

TABLE III

SUBSTANCE	1933 TO 1938						1938 TO 1943					
	DILUTION	TOTAL TESTED	PERCENTAGE POSITIVE				DILUTION	TOTAL TESTED	PERCENTAGE POSITIVE			
			+	++	+++				+	++	+++	
Cat Dander	.001	397	5	6	0	2.8	.001	772	17	8	0	3.2
Dog Dander	.001	437	11	1	0	2.7	.001	774	18	1	0	2.4
Horse Dander	.001	435	12	7	4	5.3	.001	772	22	10	6	4.9
Buckwheat	.01	440	16	4	3	5.2	.01	877	35	5	3	4.9
Egg	.001	356	10	2	2	3.9	.001	693	20	8	2	4.3
Goat Dander	.1	356	153	53	7	59.8	.01	706	196	58	8	37.1
Sheep's Wool	.1	442	100	12	1	25.5	.05	337	41	2	2	13.1

as shown in Table III extracts like cat, dog, or horse dander or even stable foods like egg white or buckwheat, containing large amounts of easily extractable excitant can be readily standardized by the nitrogen method; it is not surprising, therefore, that over a ten-year period constant nitrogen values and a uniform percentage of positive skin reactions were obtained for different lots. On the other hand, extracts containing weak allergens such as goat dander and sheep's wool gave high percentages of positive reactions, many of a nonspecific character. Dilution of these extracts, however, with consequent decrease in nitrogen values was followed by a lessened percentage of positive reaction as indicated in Table III. Here, in contrast to the fruits and vegetables, the nitrogen method still seemed to be of value in standardization.

It was evident, therefore, that neither the nitrogen nor the simple dilution method was entirely satisfactory for fruit or vegetable extracts because of the high percentage of positive reactions obtained with the former method and the inconstancy of the nitrogen content method. In 1943, therefore, following the ten-year period of study, we adopted the biologic assay method of standardization for the fruit and vegetable group. As already indicated, each new lot of extract was tested first on nonallergic controls in varying dilutions and later on known sensitive patients. The dilution giving negative reactions in the controls was used as the routine testing concentration with the next higher concentration for purposes of retesting.

The practicability of this method is indicated by the results of the comparative study with twelve commonly used food extracts procured from four other clinics. Table IV shows the results obtained with six of the extracts used, the first four being selected because they showed such variation. It is to be remembered that the extracts from our own clinic (No. 5) had been standardized previously by the method of biologic assay and were the extracts used by us routinely in testing. The extracts from Clinics 1 to 4, inclusive, likewise represented those used routinely in testing and were standardized both by dilution and protein nitrogen methods.

It is to be noted from Table IV that the extracts from Group 1 were the only ones which compared with those of our own clinic whereas the other three all showed a fairly high degree of nonspecificity. Such extracts, when used for routine testing, must necessarily provoke some

TABLE IV

CLINIC SOURCE	ASPARAGUS			WHITE POTATO			SPINACH			TOMATO			PINEAPPLE			CHEESE		
	NEG.	±	+	NEG.	±	+	NEG.	±	+	NEG.	±	+	NEG.	±	+	NEG.	±	+
I	18	2	0	18	1	1	17	3	0	18	2	0	19	1	0	20	0	0
II	12	6	2	14	6	0	4	11	5	6	10	4	17	3	0	14	6	0
III	9	7	4	16	4	0	15	5	0	16	3	1	15	5	0			
IV	10	8	2	15	4	1	16	3	1	14	6	0	16	4	0	13	7	0
V	16	4	0	18	1	1	16	3	1	17	2	1	17	3	0	20	0	0
T. U. H.																		

false positive reactions, which might have been avoided by proper preliminary testing on nonallergic controls.

DISCUSSION

The results of our studies have served to emphasize the need for more satisfactory methods of standardizing certain allergenic extracts, notably those of fruits and vegetables. Extracts containing large amounts of easily extractable excitant, like pollens, danders, or certain foods, lend themselves to ready standardization either by the nitrogen method or by simple dilution because of the character of the original material used for extraction. Those substances containing small amounts of allergen and larger amounts of extraneous material like sheep's wool or goat dander also can be standardized in similar fashion and sufficiently diluted to eliminate the nonspecific reactions. On the other hand, our studies indicate that neither the nitrogen content determination nor simple dilution method is adequate in standardizing extracts of many fruits and vegetables. This is not the fault of the method of standardization but is due to the variable nature of the raw material being extracted. Most of the fruits and vegetables contain relatively small amounts of extractable allergen, which may escape during the process of extraction and be replaced by a larger amount of extraneous material that may provoke nonspecific or irritating reactions. In the case of certain fruits, it has already been demonstrated[1, 2] that they may contain a labile antigen which, for some unknown reason, undergoes fairly rapid deterioration with consequent loss of allergenic activity. Extracts prepared in the usual manner from such fruits, therefore, may be quite inert and valueless for testing purposes.

In the light of these facts and experience, one might naturally question the advisability of continuing to employ those extracts for skin testing which are either likely to give nonspecific reactions or are so inert as to be incapable of giving a positive reaction even in the presence of clinical sensitivity. The continued use of such extracts would seem only to increase a growing skepticism not only among allergists but among the profession at large as to the value of skin tests with food extracts. Many already feel that information as to allergy to such foods would be furnished better by the clinical history or diet trial than by the conflicting results of the skin tests. It would seem preferable under the circumstances for the allergists either as a group or through their Standards Committee, to select for routine testing those food extracts which are known to be potent and to give specific reactions, for example, cereals, meats, fish, nuts and certain vegetables. All extracts known to be inert (e.g., certain fruits) or to seldom show positive reactions should be eliminated from the testing list. All others should be employed only under special circumstances when warranted by clinical history and not routinely. In either case, until we know more about the nature of the excitant and until more satisfactory methods of prep-

aration are devised, it would be advisable that each new lot of food extract be standardized by biologic assay as already described. This will serve at least to make certain that any positive reactions thus obtained on allergic patients are immunologically specific (that is, immunologic positives) even though they do not necessarily have to be clinical positives as well. Our present testing procedures, particularly as applied to food extracts, need intensive study to improve their specificity. Likewise, our skin testing list especially should undergo sharp revision, a sort of modern streamlining, if it is to retain the confidence of the profession and dispel a growing feeling that it is an overrated and abused portion of the diagnostic armamentarium in the study of the allergic patient.

SUMMARY

1. Different lots of food extracts, notably the fruits and vegetables prepared under standard conditions, were found to vary considerably in nitrogen content, apparently due to the inconstancy of the raw material.

2. The allergenic activity of such extracts seemed to bear no constant relationship to their nitrogen content.

3. The elimination of certain extracts which either are inert or give reactions of questionable value is suggested.

4. The advantages of the biologic assay method of standardizing such extracts are set forth.

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Original Articles

AIR-BORNE FUNGI IN ALLERGIC DISEASE

I. A New Method of Preparation of Mold Extracts; Incidence of Skin Reactions With Mold Extracts

A Preliminary Report

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THE high incidence of respiratory allergic diseases encountered at this hospital stimulated the study of atmospheric mold spores and their relationship to the allergic state of individuals suffering from hay fever, vasomotor rhinitis, and bronchial asthma.[1]

The exact part played by mold spores in the etiology of allergic diseases has not been definitely established, although there is evidence that they act as other air-borne allergens (pollens) in producing allergic symptoms. Since the early work of van Leeuwen and others,[2] Cadham,[3] Bernton,[4] and Hopkins and his co-workers,[5] the relationship of mycology and allergy has been studied extensively, especially by Prince and his co-workers,[6-9] Feinberg and his associates,[10-13] Pratt,[14] Harris,[15-18] and others. Morrow and Lowe[19] have recently reviewed the literature on this subject, pointing out the experimental evidence for the allergenic activity of molds and also the sources of the various molds. Harris[18] demonstrated that allergic nasal symptoms could be readily induced by the inhalation of mold spores in allergic individuals giving positive skin tests to mold extracts. Vaughan[20] pointed out that the postulates of Cooke and Thomen have been fulfilled by molds, and thus that molds are true allergens.

The study of the clinical significance of molds as etiological agents in allergic disease was started in the fall of 1943. This presentation is a preliminary report of the reaction by skin tests to mold extracts of Army Air Forces personnel, composed chiefly of aviation cadets between the ages of 18 and 27 years, and the method of preparation of mold extracts not requiring a ball mill.

PREPARATION OF EXTRACTS

Molds encountered in an atmospheric survey conducted at this hospital[23] were subcultured and maintained as stock culture from which extracts were prepared.

The unavailability of a ball mill necessitated the search for a method a preparation of mold extracts without the need of such an apparatus.

The mold selected for extraction was grown in quantity on a broth medium in a series of flasks. A modified Waksman's acid glucose-pep-

TABLE I

SUMMARY OF CASES REACTING POSITIVELY BY SKIN TESTS WITH MOLD EXTRACTS (ALTERNARIA, HORMODENDRUM, SPONDYLOCLADIUM, HELMINTHOSPORIUM) IN THE ALLERGY CLINIC, AAF REGIONAL HOSPITAL, SAN ANTONIO AVIATION CADET CENTER, SAN ANTONIO, TEXAS, OCT. 20, 1943, TO JAN. 20, 1944

CASE	DATE	DIAGNOSIS	EOSINOPHILES (%)	SKIN REACTIONS TO MOLD EXTRACTS					OTHER SIGNIFICANTLY POSITIVE SKIN REACTIONS
				POOLED MOLDS	ALTERNARIA	HORMODENDRUM	SPONDYLOCLADIUM	HELMINTHOSPORIUM	
ERM	10/20/43	Migraine	0	+	+				Pollens
PRJ	10/22/43	Hay fever	0	+	+				Pollens
WEW	10/23/43	Vasomotor rhinitis	0	+	+				None
JEN	10/25/43	Hay fever and vasomotor rhinitis	25	+	+				Pollens
GSA	10/27/43	Hay fever	2	+	+				Pollens
PDR	10/27/43	Asthma and vasomotor rhinitis	20	+	+				Pollens, inhalants, and foods
MEN	10/28/43	Vasomotor rhinitis	10	+	+				Pollens and inhalants
WRD	10/28/43	Hay fever	0	+	+				Pollens
GFP	10/29/43	Vasomotor rhinitis	5	+	+	+			Inhalants
HRC	10/30/43	Hay fever and vasomotor rhinitis	60	+	+	+			Pollens
VHR	11/ 1/43	Vasomotor rhinitis	60	+	+	-			None
VNM	11/ 1/43	Hay fever	15	+	+	+			Pollens
JDA	11/ 5/43	Asthma	0	+	+	-			Pollens and inhalants
BJW	11/ 6/43	Hay fever	0	+	+	-	-		None
ABE	11/11/43	Asthma and vasomotor rhinitis	5	+	+	+	+		Pollens
AJB	11/12/43	Vasomotor rhinitis	70	+	+	-	+		Pollens
CJB	11/13/43	Asthma and vasomotor rhinitis	15	+	+	+	-		None
SWH	11/15/43	Asthma	1	+	+	-	+		None
BJS	11/16/43	Vasomotor rhinitis	20	+	+	+	+		None
HAM	11/17/43	Hay fever	5	+	+	-	+		Pollens and inhalants
HLW	11/18/43	Asthma and vasomotor rhinitis	20	+	+	-	+		Pollens and inhalants
SEE	11/18/43	Asthma and hay fever	15	+	+	-	+		Pollens
HJC	11/19/43	Vasomotor rhinitis	20	+	+	+	+		None
DLC	11/22/43	Hay fever and vasomotor rhinitis	80	+	+	-	+		Pollens
CGK	11/23/43	Hay fever	5	+	+	+	+		Pollens
BFH	11/23/43	Hay fever	10	+	+	+	+		Pollens and inhalants
DRP	11/23/43	Hay fever and vasomotor rhinitis	40	+	+	+	+		Pollens and inhalants
VRN	11/24/43	Asthma and vasomotor rhinitis	25	+	+	+	+		Pollens and foods
ORE	11/27/43	Hay fever	15	+	+	+	+		Pollens and inhalants

+ = Positive reactions with pseudopods.
- = Negative reactions.

TABLE I—CONT'D

CASE	DATE	DIAGNOSIS	EOSINOPHILES (%)	SKIN REACTIONS TO MOLD EXTRACTS					OTHER SIGNIFICANTLY POSITIVE SKIN REACTIONS
				POOLED MOLDS	ALTERNARIA	HORMODENDRUM	SPONDYLOCLADIUM	HELMINTHOSPORIUM	
CRC	12/ 1/43	Allergic dermatitis	0	+	+	+	+		Pollens, inhalants, and foods
MKW	12/ 4/43	Hay fever and vasomotor rhinitis	15	+	+	-	+		Pollens and inhalants
MJR	12/ 6/43	Vasomotor rhinitis	5	+	+	+	+		Inhalants and foods
PBE	12/ 6/43	Asthma and vasomotor rhinitis	30	+	+	+	+		Inhalants
CEA	12/ 8/43	Asthma and vasomotor rhinitis	30	+	+	-	+		Pollens and inhalants
BJP	12/ 9/43	Vasomotor rhinitis	30	+	+	-	+		None
CJT	12/10/43	Vasomotor rhinitis	0	+	+	-	+		None
HVF	12/10/43	Vasomotor rhinitis	80	+	+				Pollens and inhalants
RBL	12/11/43	Conj. and vasomotor rhinitis	35	+	+	-	+		Pollens
IKD	12/13/43	Hay fever and vasomotor rhinitis	25	+	+	-	+		Pollens
CJB	12/14/43	Asthma and vasomotor rhinitis	20	+	+				Pollens
MRF	12/14/43	Vasomotor rhinitis	35	+	+	+	+		None
SJC	12/14/43	Asthma and vasomotor rhinitis	5	+	+	+	+	+	Pollens and inhalants
WFW	12/14/43	Asthma	5	+	+	-	+	+	None
ARB	12/18/43	Vasomotor rhinitis	25	+	+	-	-		Inhalants and foods
WCN	12/18/43	Urticaria		+	+				None
SWM	12/21/43	Vasomotor rhinitis	40	+	+	+	+	+	Inhalants
WTR	12/23/43	Vasomotor rhinitis	25	+	+	-	+	+	None
SJA	1/ 5/44	Vasomotor rhinitis	5	+	+	+	+	-	Pollens
QCO	1/ 5/44	Vasomotor rhinitis	35	+	+	+	+	+	Inhalants
SEC	1/ 6/44	Asthma	15	+	+	-	+	+	None
ACM	1/ 6/44	Asthma	25	+	+	+	+	+	None
IWW	1/13/44	Hay fever	5	+	+	+	+	+	Pollens
BCB	1/14/44	Hay fever	5	+	+	-	+	+	Pollens
VOH	1/14/44	Vasomotor rhinitis	15	+	+	-	+	+	None
FDS	1/18/44	Vasomotor rhinitis	35	+	-	-	-	+	Inhalants
EMW	1/20/44	Asthma	0	+	-	-	-	+	Inhalants
MRM	1/20/44	Vasomotor rhinitis	80	+	+	-	-	+	None

tone broth[21] was used for this purpose and most of the molds grew well on it. Since a wide surface was desired for the development of the pellicle, one-liter Erlenmeyer flasks containing 200 to 250 c.c. of broth proved the most practical and economical. The selected fungus was checked for purity, inoculated to a series of four to six flasks, and incubated in the dark at room temperatures. The time required for development generally varied from two to four weeks depending on the type of mold and the environmental conditions. The purity of the cul-

tures in all of the flasks was checked prior to their removal for extraction.

Each pellicle was lifted carefully from the surface of the medium with sterile instruments, the broth was poured off, and the felt was folded so that the upper surface was to the inside; it was then transferred to a funnel containing sterile filter paper. The pellicle was washed several times with sterile normal saline. The washed pellicle in the wet state was then ground in the sterilized chamber of a Universal food blender in the presence of sterile modified Coca's fluid (preservative omitted) and transferred to a sterile one-liter Erlenmeyer flask for a forty-eight-hour period of *germination*. The extraction ratio was 20:1 and was based on the estimated dry weight of the pellicles. The usual step of drying the pellicles was omitted since it was thought that desiccation might affect the viability of the spores and possibly the antigenic fraction of the fungus. *The purpose of allowing a period for spore germination was to render them more permeable to the extracting fluid.* After maximum germination, phenol was added to the flask to make a final concentration of 0.5 per cent and the flask placed in the refrigerator for an additional forty-eight-hour period of extraction. The suspension was next removed from the flask, pressed to separate the liquid from the fungus mass, and passed through a Seitz filter. Then the extract was bottled aseptically and sterility tests performed.

Extracts for clinical study were prepared from cultures of alternaria, hormodendrum, spondylocladium, helminthosporium, phoma, fusarium, and penicillium species. This report embodies the clinical use of the first four of these.

SKIN TESTS

Skin tests with extracts of alternaria, hormodendrum, spondylocladium, and helminthosporium species were performed on 450 routine cases in the Allergy Clinic (358 patients with allergic diseases and 92 normal subjects) from Oct. 20, 1943, to Jan. 20, 1944.

Skin tests were performed intracutaneously, on the anterolateral surface of the upper arm, by the injection of 0.01 to 0.02 c.c. of the extracts with a tuberculin or Cooke syringe with a 26 gauge, one-fourth inch length needle.

The first mold extract used in testing was alternaria so that all patients in the series were tested with this fungus. As additional extracts were prepared, they were added to the previous ones to make a pooled extract and used as such in addition to the individual molds in skin testing.

All extracts were tested in dilution of 1:100, the pooled extract containing that dilution of each component. The reading of the skin test reactions was carried out fifteen minutes after testing. Positive reactions were interpreted only in cases of wheal formation, the diameter of which was greater than 1 cm., and whose borders were irregular or presented definite pseudopodia.

TABLE II

INCIDENCE OF SKIN REACTIONS WITH POOLED MOLD EXTRACT AND ITS COMPONENTS (ALTERNARIA, HORMODENDRUM, SPONDYLOCLADIUM, AND HELMINTHOSPORIUM)

EXTRACT	NUMBER OF PATIENTS TESTED WITH POOLED EXTRACT CONTAINING THE RESPECTIVE INDIVIDUAL MOLD	NUMBER OF PATIENTS REACTING POSITIVELY TO POOLED EXTRACTS CONTAINING THE RESPECTIVE INDIVIDUAL MOLD	NUMBER OF POSITIVE REACTIONS TO THE INDIVIDUAL EXTRACT
Pooled mold extract	358	57 (15.92%)	
Alternaria	358	57	55 (15.36%)
Hormodendrum	301	44	21 (6.97%)
Spondylocladium	259	39	33 (12.74%)
Helminthosporium	101	14	13 (12.87%)

Fifty-seven of the 358 patients with allergic disease and none of the 92 normal subjects examined and skin tested in the Allergy Clinic gave significant reactions when tested intradermally with pooled mold extracts and individual extracts of alternaria, hormodendrum, spondylocladium, and helminthosporium. This is 12.66 per cent of all cases and 15.92 per cent of the cases of allergic diseases skin tested during the three-month period. Table I summarizes the skin reactions to tests with the various mold extracts and the diagnoses, nasal eosinophiles, and reactions to other allergens.

A review of Table II shows that alternaria reacted in 55 of the 358 patients with various allergic diseases. Hormodendrum reacted in 21 of 301 instances, spondylocladium in 33 of 259 instances, and helminthosporium in 13 of 101 instances when tested intracutaneously as a routine procedure. No reactions were obtained in 92 nonallergic individuals.

Sixteen of the 57 patients reacted to mold extracts alone, 32 gave positive reactions (pseudopodia) to pollen extracts, 21 gave positive reactions to inhalant extracts (dust, animal danders, etc.), and four gave positive reactions to food extracts (Table III). These four mold extracts showed no evidence of nonspecific skin irritating properties as evidenced by the large number of negative skin reactions in patients with allergic disease and the negative reactions in all 92 normal contacts.

Fifty-four of the 57 patients having positive skin tests with mold extracts had respiratory allergy (hay fever, vasomotor rhinitis, and bronchial asthma) (Table I).

TABLE III

ASSOCIATION OF POSITIVE SKIN TESTS TO MOLD EXTRACTS WITH THOSE OF OTHER ALLERGENIC SUBSTANCES

POSITIVE REACTIONS WITH MOLD EXTRACTS	NUMBER
Mold extracts only	16
Associated with positive reactions to pollen extracts*	32
Associated with positive reactions to inhalant extracts*	21
Associated with positive reactions to food extracts*	4

*Sixteen of the cases reacted positively to pollen and inhalant and/or food extracts.

DISCUSSION

Preparation of Extracts.—The method of preparation of mold extracts for use in skin testing and injection therapy has been presented not as one superior to the use of a ball mill since extracts used in this study were not compared with ball mill extracts. On the other hand, this method of preparation is recommended for use particularly when a ball mill is not available. This method utilizes the process of *germination* in the extraction.

Skin Tests.—It is an accepted fact, as discussed by Tuft,[22] that a positive skin reaction to any allergen extract is not pathognomonic of the clinical etiological offending agent but rather denotes a past, present, or potential clinical sensitivity. On the other hand, since 15.92 per cent of patients with allergic disease from various sections of the United States reacted with markedly positive skin tests to extracts of certain molds, it is highly possible that some cases of respiratory allergic diseases were due at least in part to hypersensitivity to atmospheric mold spores particularly in certain sections of the country.

The fact that 54 of the 57 cases were cases of seasonal hay fever, perennial vasomotor rhinitis, or bronchial asthma, together with the fact that only two-thirds of the patients examined in this Clinic had respiratory allergic diseases, suggests that the positive skin tests may be of specific significance. Passive transfer studies with the individual sera would have been of greater value in determining the true nature of the skin sensitivity in these cases. Since such studies were not practical, only those reactions showing wheal with pseudopodal formations were considered "positive." If the reactions of less degree had been incorporated in the analyses, the incidence, of course, would have been higher. Likewise, if additional mold extracts had been used in testing, the incidence may have been greater.

Skin Tests and Atmospheric Mold Spore Determinations.—A three-month, daily survey of atmospheric mold spores (exposed plate method) at this hospital revealed hormodendrum to be the most commonly encountered spore.[23] Of the total count of 562 colonies, 36.9 per cent were hormodendrum although this mold extract reacted by skin test in only 6.97 per cent of patients tested. Alternaria, on the other hand, was 24 per cent of the total colony count and reacted by skin test in 15.36 per cent of the patients. This was more than twice the incidence of positive skin tests to hormodendrum. Hormodendrum reacted by skin test in the least incidence of all four mold extracts even though it was found to be the most common atmospheric mold encountered by colony count in the survey. Furthermore, the survey revealed only an occasional colony of spondylocladium and helminthosporium; yet extracts of these molds reacted by skin tests in 12.74 per cent and 12.87 per cent, respectively, of the patients tested.

Allergy and Aviation Medicine.—This subject has been discussed in

a separate report in which the manner by which candidates for aviation training were studied from an allergic standpoint was described and in which the incidence of disqualification for aviation training at this Post was stated.[1] Approximately 28 per cent of the mold skin-sensitive individuals reacted only to mold extracts, although tested with eighteen common pollens and inhalants and eighteen common food extracts. Since the skin sensitivity played an important role in determining the individual's allergic state and thereby affected his probable disposition for flying, skin testing with mold extracts was of significance in these cases by aiding in establishing the presence or absence of an allergic constitution in the same manner as skin testing with pollen extracts.

Nasal Eosinophiles.—Table I shows that 47 of the 54 patients with respiratory allergy showing skin-sensitivity to mold spores had eosinophiles in the nasal secretion, denoting tissue (nasal) reactivity in keeping with allergy. Two cytological examinations of nasal secretion were performed in each case, using Giemsa's stain. One smear was prepared by the applicator technique directly from the nose, and the other was prepared after the patient had blown his nose into wax paper as described by Hansel.[24] The eosinophiles denoted in each case in Table I was the higher estimation of eosinophiles determined by the two methods.

It must be pointed out that the percentage of eosinophiles of nasal secretion quoted in this presentation does not imply a quantitative degree of allergic activity since only approximately two hundred cells were studied in each differential count. Furthermore, it is the experience of the authors that, since the cellular elements are not distributed evenly in a routine smear of nasal secretion, accurate quantitative analysis of such is impossible. The symbols cited, however, do infer a qualitative incidence.

No attempt is made at this time to evaluate the use of mold extracts in the treatment of allergic respiratory diseases, although many patients are now receiving such therapy. Further observation will be necessary before such evaluation can be made. This study, including the use of additional mold extracts in skin testing and therapy, is being continued.

SUMMARY

1. A new method of preparation of mold extracts for use in skin testing and injection therapy in allergy is presented.

2. Fifty-seven of 358 patients with allergic disease and none of 92 "nonallergic" subjects showed marked skin reactions when tested intracutaneously with extracts of atmospheric molds (alternaria, hormodendrum, spondylocladium, and helminthosporium).

3. The relationship of positive skin tests with mold extracts and the actual mold spores encountered by survey in the vicinity of San Antonio, Texas, is discussed.

4. The practical value of skin testing with mold extracts in allergy as it applies to aviation medicine is described.

5. The incidence of eosinophiles in nasal secretion of patients skin-sensitive to mold extracts is discussed.

Addendum: Protein nitrogen determinations performed on these four mold extracts four to six weeks after preparation (kept at icebox temperature) were as follows:

Hormodendrum,	0.03	mg.	protein	nitrogen	per	cubic centimeter
Alternaria,	0.18	mg.	protein	nitrogen	per	cubic centimeter
Spondylocladium,	0.47	mg.	protein	nitrogen	per	cubic centimeter
Helminthosporium,	0.55	mg.	protein	nitrogen	per	cubic centimeter

The low incidence of positive skin tests with extract of hormodendrum mold may have been due to the low protein content.

Since this preliminary study, skin tests with mold extracts have been performed using the protein nitrogen unit. The results of this more complete study will be reported at a future date.

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Editorial

Allergy at the Crossroads

IN LESS than a generation allergy developed from practically nothing into a recognized field of medicine. Like Topsy it "just grew" and in its rapid pace acquired, together with the good, many of the less desirable features of unguided youth. On account of its imaginative appeal it has become a byword on everyone's tongue, for the laity, as pedantic slang or a smart quip, and for the physician, like neurosis of old, his scrap basket for undiagnosed ailments.

In spite of a substantial amount of sound knowledge accumulated by conscientious and conservative students there has been but meager instruction even in the better medical schools over the past twenty years; hence, recent graduates, to say nothing of older practitioners, have been without theoretical background or practical experience. Thus carelessly bandied about and frequently poorly practiced, there is little reason to wonder that clinical allergy too often has run amuck, and has found itself ranking rather low in medical circles, which it should serve in view of its real significance and worth.

Such a situation, in as recent a movement as allergy, is of course not new, unusual, nor even unexpected. As a matter of fact it is the common story in most developments and most certainly in the medical world, for, as a group, physicians are given to following fads and fancies. A new concept runs wild as a prairie fire consuming all of the ideas of which the human mind is then capable and all the available techniques until it burns itself out in unproductive work over problems which it cannot solve. One has only to recall the course of events in anaphylaxis. For more than a decade after Theobald Smith's discovery was made public in 1906, there was hardly any biologic laboratory with a pretense of being scientific that was not devoted to its study. This widespread and rather hectic activity resulted in an amazing volume of literature. Finally, from the maze of conflicting theory and data there emerged a few, but relatively few, facts and even today the real and fundamental cause of the shock reaction has not been satisfactorily settled.

Now this is exactly as it should be with any concept originating from scientific research and exemplifies "the usefulness of useless knowledge," to borrow an apt phrase from Abraham Flexner, for anaphylaxis would have ceased to attract continued scientific interest and would have become a useless biologic curiosity were it not for the fact that it became the direct progenitor of allergy.

The past twenty-five years have seen the same feverish activity in allergy as in anaphylaxis and the same era of conflicting theories and

observations which still await an answer. Now it has seemingly reached a stage of diminishing returns and insoluble problems unless, or until, further research in this and allied fields opens new and promising leads.

In spite of this, however, allergy will continue to hold the interest of the physician on account of its proved practical application in the diagnosis and treatment of disease, provided our technical procedures are corrected and improved, our allergy patients are studied as medical problems rather than used as subjects for excessive skin testing, and the diagnoses are made in conformity with good sense while management and treatment are rendered more effective.

Today allergy stands, as it were, at the crossroads. Either it must maintain a worthy and dignified place in the field of medicine or become a minor technical adjunct to the internist. It is high time, then, that those who are genuinely interested in allergy bestir themselves in its behalf. This means work—hard and honest work—in the clinic and in the laboratory. In experiments or observations for scientific papers, the results must be checked and rechecked. Each worker should be his own severest critic and should try harder to disprove his point. There has been too much jumping at conclusions and rushing into print, too many preliminary reports that are never followed by a final or conclusive one.

In practice we should have a more meticulous medical survey of the patient having or presumed to have an allergic condition; the many curious coincidences following therapeutic measures and dietetic restrictions must not be interpreted and reported as cause and effect unless supported by repeated trials. Etiological diagnoses must have the ring of sound common sense and not wishful thinking.

General improvement in the practice of allergy will come only through greater care and education. Because some still engaged in the practice of allergy had to educate themselves under that hard taskmaster, experience, is no reason to believe that self-teaching is the safest or best course. Existing knowledge in the field, which has become considerable, must be made available to all who wish to learn. It is for this reason that the American Academy of Allergy has created a Committee on Education which has undertaken a careful study of the entire field of education, undergraduate, graduate, and postgraduate. It will stimulate educational opportunities through fellowships and create varied types of courses; it will assist and sponsor those which it can recommend. Through reports or editorials it will keep readers of the JOURNAL informed as to its ideas and plans from time to time.

Only through the teaching of sound allergy practice and by study that advances our knowledge will the subject of allergy maintain its proper position as an important part of internal medicine.

—*R. A. C.*

On the Standardization of Extracts

IN THIS issue are two papers concerned with the preparation of pollen extracts. In one Berresford and Cooke[1] call attention to the preparation and preservation of pollen and to the importance of moisture in the pollen structure. Pollen that contains moisture may support the growth of molds, and there is little doubt but that molds can modify the allergenic activity of pollen just as they can modify the activity of cotton or kapok fibers. Furthermore, there is evidence that extracts made with dry pollen will be a little different from other extracts made with slightly moist pollen. Standard extracts should be made from standard pollens.

The second paper is by Tuft, Blumstein, and Wenger,[2] who discuss the difficulties of standardizing extracts of food. They propose that the biologic method is, after all, better than the chemical method.

A question which comes up is, "What are we trying to standardize?" In most instances "good" extracts are those which give positive reactions on the skin of sensitive persons and which show no reactions on the skin of normal individuals. The emphasis is on skin tests. This is well enough in many ways, but what about the treatment? It is necessarily true that the material which gives good reactions on skin test is also the material which is effective in treatment?

The question is a large one and it opens an important field of thought. For example: In the old days before Ford, the treatment of patients with horse asthma with horse dander extract was attempted, but, each time, any "reasonable" dose was prevented by the excessive local reaction. The therapeutic effect of the extract was limited by the large amount of skin-test-active material which it contained. More recently, the relation between the large amounts of pollen required to produce any measurable change in the antibodies of normal people in contrast to the tiny amounts employed in the treatment of hay fever sufferers has been commented upon by Cooke and his co-workers.[3] If there is a blocking antibody which is distinct from the skin sensitizing antibody, then there must be two antigens in the extract and their functions may well be different. Standardization will have to be different also.

For the moment, however, The American Academy of Allergy has voted "that as a temporary expedient the pollen quantity as described in published papers and in presented talks shall be explained in terms of units in which one unit is equivalent to quadruple 0 one (0.00001) milligrams of nitrogen as precipitated by phosphotungstic acid."

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3. Cooke, R. A., Loveless, M., and Stull, A.: Studies on Immunity in Type of Human Allergy (Hay Fever): Serological Response of Non-Sensitive Individuals to Pollen Injections, J. Exper. Med. **66**: 689, 1937.

—*F. M. R.*

American Academy of Allergy

ANNUAL MEETING

PRESIDENTIAL ADDRESS

ROBERT CHOBOT, M.D., NEW YORK, N. Y.

FELLOWS and Members of the American Academy of Allergy: Today the Academy celebrates its first anniversary, and it is fitting and proper that we review the activities of the past year. These will be reported in detail by the chairmen of the various committees later in the morning.

For a number of years, a merger of the two established national societies had been contemplated, by reason of the fact that their aims and ideals had been identical, and that, to a great extent, they had enjoyed a mutual membership. One year ago this merger was consummated. This was an important step forward; it has eliminated a great many duplications of membership and has prevented a duplication of activities. I am pleased to say that the members of this Academy have done everything possible to eliminate small points of difference that have arisen, and with the first difficult year behind us, I am certain that this Academy shall survive and prosper. We have achieved a degree of unity of action and purpose that a great many skeptics felt was impossible.

I should, however, be remiss in appreciation if I failed to pay tribute to the fine spirit that has characterized our officers, the Executive Committee, and the entire membership, all of whom have given freely of their time and energy. Their unstinting devotion to the Academy has been most laudable, and without it our progress would have been impossible.

I feel that great progress has been made in this Academy in one year. Let us review briefly some of the accomplishments. We have embarked on an educational program. The Committee on Education whose report you will have detailed for you shortly has been organized to promote all the phases of education in allergy. The immediate developments have been in the field of graduate teaching. We have been fortunate in establishing a national faculty which will enable us to supply teaching needs anywhere in the United States. The committee has done an excellent job, and the members of the Academy are greatly indebted to them.

The Committee on Pharmaceuticals and Medicaments started out under the leadership of the late Warren Vaughan whose sudden and untimely demise was a grievous blow to the Academy. Subsequently, your

Delivered at the First Annual Meeting of the American Academy of Allergy, New York, Dec. 12, 1944.

President was asked to serve as temporary chairman. I should like to report the work the committee has been doing.

First, the committee has become closely affiliated with the Council of Pharmacy of the American Medical Association and will act as their representative in the field of allergy. Second, the Federal Food and Drug Administration is working in close relation with this committee. As a result of our combined activities, several nostrums have been removed from the market.

I feel that the Committee on Pharmaceuticals and Medicaments should take a more active part in the collection and preservation of pollens. In the near future I believe it would be well for us to consider the idea that we arrange to collect our own pollens and see that the standards of collection are set up and supervised by governmental agencies. This will affect the quality of extracts and commercial preparations made available to the lay public and to the medical profession. I believe that the committee should establish facilities for pollen counts on a national scale. These counts should be published daily in the newspapers of the country as part of the activities of the Academy of Allergy.

One of the most important future activities of the committee should be in the field of public relations. We must, in the future, see to it that the lay public is promptly informed of the utter worthlessness of certain types of so-called cures. For example, had the Academy been in existence, the advent of vitamin C as a supposed cure would have been promptly challenged. I feel that the Academy should avail itself of publications such as *Hygeia* and the lay press, so that the public will be honestly informed as to the value of the various cures and mechanical devices. The Academy can serve and protect the lay public by so doing. In addition it should sponsor properly supervised radio programs.

May I call attention to the fact that the committee expects to serve as a subcommittee of the editorial staff of the JOURNAL in approving future advertisements of drugs to be published in THE JOURNAL OF ALLERGY. The purpose of this will be the Protection of the ill-informed physician and the public who depend on the advertisements as their source of information.

We have come a long way, but on the road ahead it is but a short step in the right direction.

Now what of the future? I should like to see further developments and advances made in the direction of education and standardization of extracts.

I believe that the teaching of allergy should be one of the main activities of the Academy. The medical student, even under a four-year course, receives comparatively little education in allergy in his undergraduate years. With the advent of the three-year medical course, teaching of allergy has been reduced to a bare minimum, and it should be our first duty to see to it that undergraduate teaching of allergy is increased as soon as the medical curriculum returns to prewar conditions.

We have had numerous short graduate courses in the past and these must be amplified. It is, in my opinion, essential that a standard curriculum be followed in order to achieve uniformity. The Academy must publish an approved list of courses and the places and the times when these courses are to be given.

There is additional need for the teaching of specialists, and I feel that there is a need for the establishment of a graduate school in allergy. This school should, if possible, be affiliated with a university and should comprise, just as any other school does, elective courses of which the more advanced students could avail themselves. The graduate school should have a library consisting not only of current books and periodicals pertaining to the subject, but also a complete series of lantern slides and motion pictures suitable for teaching purposes. Today there are a great many films and slides in the hands of our Fellows. These, or similar ones, should become the nucleus available for teaching.

This school and library should be one of the administrative activities of a paid secretary. It is not fair, nor practicable, to ask any officer of the Academy to assume these additional responsibilities. The entire program of certifying clinics and fellowships depends on the availability of an individual free to travel and devote his entire energies to this, and other tasks. A paid secretary could organize groups for courses and could serve us to the greatest advantage in organizing our teaching program. He would, of course, assume the administrative problems that in the years to come will be too much to ask of any active practitioner.

It is my opinion that, to successfully carry out its program, the Academy shall require funds. Available funds would enable us to employ a secretary. Furthermore, funds would assure our functioning most effectively as a teaching group. It would, likewise, enable the Academy to finance research fellowships at various medical centers.

Such funds could either be administered by a foundation or by a committee of the Academy. There are, of course, objections that have been raised, and must be considered. The first is that a foundation carries with it enormous responsibilities. We are of age and we cannot shirk these if we are to achieve our goal. The second objection is the danger of jealousies arising from "grants in aid." My feeling is that, if that be the prime objection, we should limit our activities for the time being to objectives other than research grants.

The means by which necessary funds are to be raised poses another problem. I can assure you, from preliminary conversations, that the money is available.

My proposal would be to obtain funds to be granted without reservation, for the purpose outlined above. Contributors should have representation on a board to enable them to become well acquainted with the way their monies are being spent. I urge the membership to give thought to this proposal, as I feel that it has merit.

The Academy can never hope to reach the size of the other large medical societies due to the fact that the number of men in allergy is limited. As a result it will be impossible to finance an extensive teaching program out of our own income.

It is, however, in the field of standardization that the Academy must direct its efforts. We have adopted a pollen unit which is expressed both in terms of protein nitrogen, total nitrogen, and weight by volume. This unit was adopted by the Executive Committee with the full knowledge that we had not settled the problem. It is true that the protein nitrogen unit is by no means the last word in pollen standardization, but, likewise, it is true that we are better off using the best standard we have and using it universally, than to continue in the present state of disorganization. It certainly should be one of our future activities to see to it that research fellowships are established in the departments of chemistry in several outstanding universities. The future work on the active principle of pollen must seek its ultimate solution this way. It is essential to have careful chemical collaboration in any such problems, but it is high time that we, as clinicians, realized our limitations; namely, that we are physicians and not research chemists, and it is the latter group to whom we must turn. As soon as the war permits, fellowships, under outstanding chemists, should be established to further investigate the problem, and I believe that in the not too distant future it will be possible to have the Academy adopt an official standard with routine methods of preparation for all allergenic extracts. It is self-evident what great progress would result from such action. Teaching courses would be more standardized and treatment doses would be uniform. The benefits accruing to the patient traveling from city to city would be enormous.

The growth and stability of our organization is assured, but we must never lose sight of the fact that one of our main functions is to be of service to our profession and to the lay public. Our activities in these directions must never be permitted to lag. In addition, we must see to it that the current knowledge in allergy is widely disseminated to the public and the profession. We should not, we cannot, and we must not allow quackery and scientific dishonesty to be associated with the name of allergy. The sooner it is made apparent to both the lay public and the profession that there are no "quick" or "one injection" cures for the allergic state, nor that there are any miraculous nostrums to cure all allergic ills, the sooner will allergy take its place among the other medical specialties.

Before closing, I should like to take this opportunity to convey to you my deep appreciation of the honor that you have bestowed upon me in permitting me to serve as your President for the past year. It has been a privilege for which I thank you sincerely.

TRANSACTIONS OF THE ANNUAL MEETING

At the business session of the Academy, held on Tuesday morning, December 12, various committee reports were made.

Upon the recommendation of the Credentials Committee, the following Members were elevated to Fellowship in the Academy:

Dr. Armand E. Cohen, Louisville, Ky.
Dr. Harry L. Rogers, Philadelphia, Pa.
Dr. Henry I. Shahon, West Roxbury, Mass.
Dr. J. Warrick Thomas, Richmond, Va.
Dr. Benjamin Zohn, Brooklyn, N. Y.
Dr. J. Harold Friedman, Cleveland, Ohio

Also, upon recommendation of the Credentials Committee, the following were elected to Membership in the Academy:

Dr. J. Mercer Anderson, Salt Lake City, Utah
Dr. Rudolph Baer, New York, N. Y.
Dr. Clarence Bernstein, Jr., Orlando, Fla.
Dr. Philip Blank, Pittsburgh, Pa.
Dr. Robert H. Chait, Fort Lawton, Wash.
Dr. Leoni Claman, New York, N. Y.
Dr. J. F. Clancy, Hammond, Ind.
Dr. William J. Colonnell, Bethesda, Md.
Dr. William V. Conn, Greensburg, Pa.
Dr. Frank G. Crandall, Los Angeles, Calif.
Dr. H. E. Diamond, Bronx, N. Y.
Dr. Manly Finkelstein, New York, N. Y.
Dr. Sidney Friedlaender, Chicago, Ill.
Dr. James Gant, Bethesda, Md.
Dr. James Gottesman, New York, N. Y.
Dr. Randolph Graham, Richmond, Va.
Dr. Leonard Horn, Fort Dix, N. J.
Dr. Stanley Insley, Detroit, Mich.
Dr. Harry Kaplan, New York, N. Y.
Dr. Cecil H. Kohn, Kansas City, Mo.
Dr. Leopole C. Lazarowitz, New York, N. Y.
Dr. Howard James Lee, Topeka, Kan.
Dr. Meyer Marks, Miami Beach, Fla.
Dr. H. I. Miller, Pittsburgh, Pa.
Dr. John H. Mitchell, Columbus, Ohio
Dr. William F. Mitchell, Columbus, Ohio

Dr. Benjamin Nozik, Cleveland, Ohio
Dr. Jose M. Quintero, Havana, Cuba
Dr. Theron G. Randolph, Chicago, Ill.
Dr. Simon Rubin, Chicago, Ill.
Dr. Richard A. Shields, Jr., Philadelphia, Pa.
Dr. David Silberman, Bronx, N. Y.
Dr. Iola Sivon, Columbus, Ohio
Dr. Charles E. Sutherland, Victoria, Australia
Dr. Harry Swartz, Fort Dix, N. J.
Dr. Richard H. Todd, Washington, D. C.
Dr. Norman Van Wezel, Montgomery, Ala.
Dr. Karl D. Way, Akron, Ohio
Dr. S. R. Zoss, Youngstown, Ohio

The report of the Credentials Committee included a recommendation, accepted by the Executive Committee, that applicants request membership forms from the secretary and that applications, when filled in, be sent directly to the Chairman of the Credentials Committee instead of to the Secretary as heretofore.

Upon recommendation of the Nominating Committee, the following officers were elected for the year 1945 by the Fellows present:

Dr. Stearns S. Bullen, Rochester, N. Y., Vice-President
Dr. Karl D. Figley, Toledo, Ohio, Secretary
Dr. Leo H. Criep, Pittsburgh, Pa., Treasurer

At this business session, a constitution was adopted by the Academy, after a few slight changes in the original form with which all the Fellows and Members had previously been supplied. According to this constitution, the Vice-President automatically becomes president, and the retiring President becomes a member of the Executive Committee. Accordingly, the following are the Academy officers for the year 1945:

Dr. Oscar Swineford, Jr., President
Dr. Stearns S. Bullen, Vice-President
Dr. Karl D. Figley, Secretary
Dr. Leo H. Criep, Treasurer

Executive Committee

Dr. Matthew Walzer, Brooklyn, N. Y.
Dr. Milton B. Cohen, Cleveland, Ohio
Dr. Robert A. Cooke, New York, N. Y.
Dr. Samuel M. Feinberg, Chicago, Ill.
Dr. Robert Chobot, New York, N. Y.
and the four officers above named.

New committee appointments and changes for the ensuing year are as follows:

Advisory Committee

Dr. Robert A. Cooke, Chairman, six-year term
Dr. Harry L. Alexander, five-year term
Dr. Francis A. Rackemann, four-year term
Dr. Leslie Gay, three-year term
Dr. Harry Huber, two-year term
Dr. George Piness, one-year term

Nominating Committee

Dr. Charles H. Eyermann, Chairman
Dr. Alan G. Cazort
Dr. Mary H. Loveless

Program Committee

Dr. Oscar Swineford, Chairman
Dr. Harry L. Alexander
Dr. Edmund L. Keeney
Dr. Wyndham B. Blanton

Dr. Robert Lamson was appointed to the Credentials Committee, Dr. Theodore L. Squier to the Merits Committee, and Dr. Frank Simon to the Committee on Pharmaceuticals and Medicaments. Dr. Samuel Feinberg was appointed chairman of the Local Committee on Arrangements for the second Annual Meeting in Chicago. He will make further committee appointments.

The following motion was passed. The American Academy of Allergy recommends that, as a temporary expedient, when pollen quantity is described in published papers and in oral presentations it be expressed in terms of units in which one unit is equivalent to 0.00001 mg. of pollen nitrogen as precipitated by phosphotungstic acid.

The business session of the Academy concluded with the announcement that the next meeting will be held in Chicago, at the Palmer House, on December 10 and 11, 1945.

Report of the Meeting of the Advisory Committee

The meeting was called to order at 10 A.M. All members of the Committee were present. Aspects of certification in allergy were discussed. It seemed to be the consensus of opinion of the Committee that certification by the American Board of Internal Medicine as now in operation was entirely satisfactory, that the bars should not be let down nor should any attempt be made to lower the standards as a result of the complaints that have been made. These complaints seem to be decreasing rather than increasing in number.

A letter from Dr. C. Anderson Aldrich of the American Board of Pediatrics was read. This Board is apparently favoring certification in allergy for those certified in pediatrics, using the same system and the same Advisory Committee that is now operating with the American Board of Internal Medicine. However, no definite action has yet been taken.

The approval of clinics, which formerly was a function of this Committee, has definitely been eliminated. It is the general consensus of opinion that the approval of clinics as heretofore carried out, has not been satisfactory and should not be resumed until some proper method of clinic investigation can be secured.

During the past year some eighteen or twenty names have been submitted to this Advisory Committee by the American Board of Internal Medicine for their approval for examination in allergy. In approving these men it is our feeling that we should still adhere to the rules that were formulated by the Committee as published in The JOURNAL OF ALLERGY **14**: 91, 1942.

No further matters were brought before the Committee for discussion and the meeting was adjourned at 11:40 A.M.

ROBERT A. COOKE, CHAIRMAN

REPORT OF THE COMMITTEE ON PHARMACEUTICALS AND MEDICAMENTS

Report on Nakamo-Bel.—A clinical study was recently completed of the effect of Nakamo-Bel in allergic conditions. This study was made upon the request of the Federal Security Agency, Food and Drug Administration to the Committee on Pharmaceuticals and Medicaments of the American Academy of Allergy.

The drug was tested clinically by five independent observers in twenty-two cases of typical autumnal hay fever and in seven cases of bronchial asthma.

The tablets used for these twenty-nine cases were supplied by the Food and Drug Administration and were administered as recommended by the manufacturer. No symptomatic benefits were noted by any of the five observers in any patient. The detailed reports were forwarded to the office of the Food and Drug Administration.

ROBERT CHOBOT, CHAIRMAN

NOTES

Word has been received of the sudden death of Dr. Aaron Brown. Appropriate notice will appear in the next issue of the JOURNAL.

Dr. Mary Loveless was the recipient of the first Marcelle Award of $350, presented by the Seventh Annual Forum in Pittsburgh, January 26, for her attempt to correlate thermostabile antibody with clinical response in hay fever. The second Marcelle Award of $150 was granted to Dr. Charles F. Code, of the Mayo Foundation, for his studies of the role of histamine in the production of anaphylactic and allergic reactions. Honorable mention went to Dr. Arnold Rich, of Johns Hopkins Medical School, and his associates, for their work on serum sickness and periarteritis nodosa, and to Dr. Frank Simon, of Louisville, for his studies of the allergens in human dander. The annual gold medal was presented to Dr. Milton J. Rosenau, Professor of Epidemiology, University of North Carolina.